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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1650175> since 2019-09-04T11:55:16Z

Published version:

DOI:10.1136/gutjnl-2016-313372

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This is the author's final version of the contribution published as:

Barault L; Amatu A; Siravegna G; Ponzetti A; Moran S; Cassingena A; Mussolin B; Falcomatà C; Binder AM; Cristiano C; Oddo D; Guarrera S; Cancelliere C; Bustreo S; Bencardino K; Maden S; Vanzati A; Zavattari P; Matullo G; Truini M; Grady WM; Racca P; Michels KB; Siena S; Esteller M; Bardelli A; Sartore-Bianchi A; Di Nicolantonio F. Discovery of methylated circulating DNA biomarkers for comprehensive non-invasive monitoring of treatment response in metastatic colorectal cancer. GUT, Oct 5, 2017. DOI: 10.1136/gutjnl-2016-313372. [Epub ahead of print]

The publisher's version is available at:

<http://gut.bmj.com/content/early/2017/10/05/gutjnl-2016-313372>

When citing, please refer to the published version.

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Discovery Of Methylated Circulating DNA Biomarkers For Comprehensive Non-Invasive Monitoring Of Treatment Response In Metastatic Colorectal Cancer.

Ludovic Barault,^{1,2,#} Alessio Amatu,³ Giulia Siravegna,^{1,2,4} Agostino Ponzetti,⁵ Sebastian Moran,⁶ Andrea Cassingena,³ Benedetta Mussolin,² Chiara Falcomata,^{1,2} Alexandra M Binder,⁷ Carmen Cristiano,⁵ Daniele Oddo,^{1,2} Simonetta Guarrera,⁸ Carlotta Cancelliere,² Sara Bustreo,⁵ Katia Bencardino,³ Sean Maden,⁹ Alice Vanzati,⁴ Patrizia Zavattari,¹⁰ Giuseppe Matullo,⁸ Mauro Truini,³ William M Grady,^{9,11} Patrizia Racca,⁵ Karin B Michels,⁷ Salvatore Siena,^{3,12} Manel Esteller,^{6,13,14} Alberto Bardelli,^{1,2} Andrea Sartore-Bianchi,^{3,*} Federica Di Nicolantonio^{1,2,*,#}.

Corresponding authors

* Co-senior authors

Author affiliations

1 Department of Oncology, University of Torino, Torino, Italy

2 Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Italy

3 Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milan, Italy

4 FIRC Institute of Molecular Oncology (IFOM), Milano, Italy

5 Colorectal Cancer Unit, Medical Oncology Division 1, AOU Citta della Salute e della Scienza, San Giovanni Battista Hospital, Turin, Italy

6 Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute, L'Hospitalet, Barcelona, Spain

7 Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, California, USA

8 Department of Medical Sciences, Italian Institute for Genomic Medicine – IIGM/ HuGeF, University of Torino, Torino, Italy

9 Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

10 Department of Biomedical Sciences, Unit of Biology and Genetics, University of Cagliari, Cagliari, Italy

11 Department of Medicine, University of Washington School of Medicine, Seattle, Washington, USA

12 Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

13 Department of Physiological Sciences II, School of Medicine, University of Barcelona, Barcelona, Catalonia, Spain

14 Institutio Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain

Corresponding authors:

Ludovic Barault, PhD or Dr Federica Di Nicolantonio, PhD

Department of Oncology, University of Torino

Candiolo Cancer Institute – FPO, IRCCS

Strada Provinciale 142, Km 3.95

10060 Candiolo, Torino, Italy

Phone: +39-011-9933523

Fax: +39-011-9933225

(e-mail: ludovic.barault@unito.it; federica.dinicolantonio@unito.it)

Short running head: Circulating methylated biomarkers for colorectal cancer monitoring

What is already known about this subject?

- ▶ Cancer mutations (eg, *BRAF*, *KRAS*, *TP53*), in cell-free circulating DNA could be used as markers of treatment response or disease relapse in patients with colorectal cancer but require prior knowledge of individual gene variants.
- ▶ DNA methylation alteration is a common early event in colorectal carcinogenesis and is detectable in cell-free circulating DNA; hence it was studied for early detection (eg, *SEPT9*) or tumour monitoring (eg, *NPY*, *WIF1*).
- ▶ Previous identification of methylated markers most often relied on assessment of few samples with low genome coverage methods and might have omitted important putative cancer-specific markers.

What are the new findings?

- ▶ Genome-wide DNA methylation analysis from stroma-free colorectal cancer cells identifies highly prevalent and specific methylated loci (*EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1* and *MSC*).
- ▶ Assessment of methylated markers in cell-free circulating DNA allows non-invasive monitoring of disease.
- ▶ DNA shedding of methylated markers is not impaired by treatment type or duration.
- ▶ Methylation changes over time correlate with tumour response evaluated by CT scan in patients with metastatic colorectal cancer treated with chemotherapy or targeted agents.

How might it impact on clinical practice in the foreseeable future?

- ▶ This panel of methylated markers was able to monitor tumour burden in patients with colorectal cancer treated with different conventional treatment regimens, including chemotherapy, anti-angiogenic agents and targeted agents. Liquid biopsy based on methylated markers could be coupled with imaging to improve timely therapeutic changes. This approach could also be useful for early pharmacodynamics assessments in clinical trials.

Abstract

Abstract

Objective Mutations in cell-free circulating DNA (cfDNA) have been studied for tracking disease relapse in colorectal cancer (CRC). This approach requires personalised assay design due to the lack of universally mutated genes. In contrast, early methylation alterations are restricted to defined genomic loci allowing comprehensive assay design for population studies. Our objective was to identify cancer-specific methylated biomarkers which could be measured longitudinally in cfDNA (liquid biopsy) to monitor therapeutic outcome in patients with metastatic CRC (mCRC).

Design Genome-wide methylation microarrays of CRC cell lines (n=149) identified five cancer-specific methylated loci (EYA4, GRIA4, ITGA4, MAP3K14-AS1, MSC). Digital PCR assays were employed to measure methylation of these genes in tumour tissue DNA (n=82) and cfDNA from patients with mCRC (n=182). Plasma longitudinal assessment was performed in a patient subset treated with chemotherapy or targeted therapy.

Results Methylation in at least one marker was detected in all tumour tissue samples and in 156 mCRC patient cfDNA samples (85.7%). Plasma marker prevalence was 71.4% for EYA4, 68.5% for GRIA4, 69.7% for ITGA4, 69.1% for MAP3K14-AS1% and 65.1% for MSC. Dynamics of methylation markers was not affected by treatment type and correlated with objective tumour response and progression-free survival.

Conclusion This five-gene methylation panel can be used to circumvent the absence of patient-specific mutations for monitoring tumour burden dynamics in liquid biopsy under different therapeutic regimens. This method might be proposed for assessing pharmacodynamics in clinical trials or when conventional imaging has limitations.

Introduction

Tumours release fragments of nucleic acids into circulation, which could provide a minimally invasive surrogate for tissue biopsy as well as offering the opportunity of serial sampling over time.^{1 2} Recent liquid biopsy studies have evaluated tumour-specific mutations or gene copy number changes in cell-free circulating DNA (cfDNA) derived from patients with digestive tract cancer for early diagnosis³ and for monitoring the emergence of disease relapse.^{1 4–7} This approach usually relied on alterations in oncogenic drivers such as *RAS* and *BRAF*—up to 50% of the colorectal cancer (CRC) population—or mutations within oncosuppressor genes (usually with no variant hotspots), therefore requiring specific assay design for each mutation. Studies mainly focused on follow-up after surgery^{5 6 8} or on patients with CRC treated with epidermal growth factor receptor (EGFR) targeted therapies,^{4 9 10} in which detection of oncogenic *RAS* alterations is associated with impaired treatment response. Changes in epigenetic patterns are often associated with alterations in physiological or pathological conditions triggering cell death and release of DNA with specific epigenetic marks,¹¹ which prompted exploring liquid biopsy tests as a surrogate of liver fibrosis severity in individuals affected by non-alcoholic fatty liver disease.¹² Changes in DNA methylation are considered an early event in carcinogenesis¹³ and have already been proposed for early tumour detection in different settings (including CRC), allowing non-invasive population screening using stool^{14 15} or blood.^{16–21} The Food and Drug Administration recently approved the first blood test exclusively based on *SEPT9* methylation, which might lead to improvements in CRC screening uptake.^{16 17} Here we propose the evaluation of DNA methylation markers in cfDNA, not as an early detection method, but rather as a non-invasive treatment-monitoring assay. Review of methylation markers for early detection of CRC identified genes which were highly tumour specific (not found in normal adjacent mucosa), but rarely validated in large cohorts.²² While using innovative approaches and being very

informative, studies which aimed at identifying cancer-specific methylated markers usually relied on platforms with low genomic coverage,²¹ or small sample datasets,²³ or exclusively assessed patient tissue^{18 24} which might have been partially infiltrated with stroma. We therefore employed genome-wide assessment of DNA methylation in a large collection of 149 CRC cell lines and identified a fivegene panel that was validated in tissue and cfDNA from patients with metastatic CRC (mCRC) using digital PCR-based assays (methyl-BEAMing^{19 25}). Longitudinal assessment of this panel in cfDNA was performed to monitor disease burden in patients with mCRC over diverse treatment courses.

Materials and methods

Additional details are available in online supplementary file 1.

Cell lines and genome-wide DNA methylation data processing and retrieval

A collection of 149 cell lines of intestinal origin (see online supplementary file 2A) was assembled, as described.²⁶ Infinium HumanMethylation450 BeadChip arrays were prepared according to manufacturer's protocol. Cell line methylation profiles are available on Gene Expression Omnibus ([http:// www. ncbi. nlm. nih. gov/ geo/](http://www.ncbi.nlm.nih.gov/geo/); accession number: GSE86078). After removing ulcerative colitis cases, cancer unrelated normal mucosa samples from GSE32146 were used as a control set. GSE41169 was employed as a blood control dataset (ie, healthy donors). GSE42752²³ was used as a test cohort for establishing an in silico validation threshold. Level 1 data from the TCGA colorectal adenocarcinoma (COAD/READ) ([https:// gdc- portal. nci. nih. gov](https://gdc-portal.nci.nih.gov)) were used for the in silico validation. Further validation in normal tissues was performed using GSE48684.²⁴

Data preprocessing and marker discovery analysis

All raw data (IDAT files) were preprocessed in R Bioconductor (minfi²⁷). Probe signal was removed when the detection p value was above 0.05, or >1% of the dataset contained no

data, or if probes contained single nucleotide polymorphisms,²⁸ demonstrated sexual dimorphism²⁹ or were located on sex chromosomes. Our dataset was then merged with the other publicly available cohorts. Differential methylation analysis was performed at probe (lmFit from limma; adjusted $p \leq 1 \times 10^{-35}$; minimum delta beta-value of 0.8) or region level (bumphunter from minfi; threshold=0.8; regions represented by at least two probes with $L \geq 2$). Methylation in leucocytes was excluded (GSE41169; maximum beta-value allowed=0.1) to minimise the risk of false positivity in blood tests. Differentially methylated probes were limited to those overlapping differentially methylated regions, and 'liquid biopsy' assessable loci were defined as regions represented by at least two selected probes distant of a maximum 150 bp, the average fragment size reported in cfDNA³⁰ and not located in centromeres or telomeres (see online Supplementary file 2B). Receiver operating characteristic analyses were performed in GSE42752 with the pROC package in R Bioconductor³¹ to establish thresholds, considering normal and adjacent mucosa as positive outcome and cancer as negative; only loci showing a threshold below 0.35 were kept. Each threshold was used to stratify the TCGA COAD/READ cohort, defining a positive predictive value (PPV) and negative predictive value (NPV) for discriminating normal adjacent from tumour tissue. Normal healthy and peritumoural samples from GSE48684²⁴ were also controlled for absence of methylation above the threshold. All data used for the in silico analyses were from DNA derived from fresh frozen samples.

Methylation and genetic alteration assays for cfDNA evaluation

Assays were designed and optimised as described²⁵ to allow methylation independent amplification. Full details are given in online supplementary file 2C and 3. In silico validation was performed using solely the probes which were located within the amplicon; results for the five markers of interest are displayed in online supplementary file 4. Evaluation of genetic alterations (*KRAS*, *BRAF* mutations and *MET* gene copy number) in

cfDNA was performed as reported.^{4 32} Assays, commercially available (Bio-Rad), are listed in online supplementary file 2C.

Tissue collection and DNA isolation

Formalin-fixed paraffin-embedded (FFPE) tissues originated from two different cohorts. The first consisted of macro-dissected tumour and normal adjacent tissues (n=31 cases; originating from several local hospitals), which were controlled for tumour purity and assembled at Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda (Milan, Italy) and from which DNA was newly extracted (average DNA concentration: 689 ng/μL (59–1844)). A second dataset of independent FFPE tumour tissues (n=51) was assembled from remaining DNA extracted during the enrolment of the DETECT-01 (EudraCT number 2011-002080- 21)³³ or TEMECT (EudraCT number 2012-003338-17)³⁴ trials (average DNA concentration: 163 ng/μL (10–776)).

Plasma collection and DNA isolation

De-identified plasma samples (average volume 1.5 mL (0.8–1.9)) from self-declared healthy donors (n=50) were purchased from the Brigham and Women's Hospital specimen bank (Boston, USA). One hundred and eighty-two mCRC cases were retrospectively enrolled in the study. One hundred and thirty-seven cases were selected for plasma time-points based on blood sample availability at a time when patients were presenting radiological evidence of disease. The remaining 45 cases were treatment baseline specimens, selected for availability of longitudinal follow-up samples (additional total of 132 longitudinal samples). The plasma volume obtained from patients with mCRC for the study was on average 1 mL (0.5–1.4). Summary of the clinicopathological features of the two cohorts can be found in online supplementary file 2D and patient clinical features are presented in online supplementary file 2E. mCRC plasma samples were collected at Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda (Milan, Italy) or at San Giovanni Battista Hospital (Turin, Italy). The study was conducted according to Good

Clinical Practices and was approved by the local ethics committee. Circulating DNA was extracted as previously described²⁵ from 1 mL of plasma or less due to limited amount availability.

Statistics and data analyses

All methylation microarray analyses and figures were generated in R Bioconductor as previously mentioned. Prevalence and longitudinal representations were assembled in GraphPad. Wilcoxon test (for matched tissues analysis), Mann-Whitney U test and Kruskal-Wallis test (for group prevalence analyses) and Mantel-Cox log rank test (for survival analysis) were performed in GraphPad. Scatter matrix for marker correlation was obtained using OriginPro 2016 (OriginLab). All expressed p values were calculated with two-tailed tests and were considered significant when $p \leq 0.05$, unless otherwise specified.

Results

Marker selection and assay optimisation

Differential methylation analysis between CRC cell lines and normal mucosa identified 162 CpG dinucleotides representative of a larger genomic region controlled to be unmethylated in blood cells which represent the main contaminant source of cfDNA (figure 1, Supplementary file 2B and 5). Among the markers previously proposed for liquid biopsy assessment of CRC, *SEPT9* was discarded for few differentially methylated probes too distant from each other; *WIF1* was removed because of low differential methylation between normal and tumour tissue; while *NPY* demonstrated positivity in normal mucosa samples and blood cells (see online supplementary file 6). Thirty- nine loci defined by 93 probes, were selected as liquid biopsy assessable regions (see online supplementary file 5). An in silico validation (see online supplementary file 4), confirmed that the selected methylated loci were cancer specific and not a consequence of cell line establishment and identified six loci (five genes: *EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*) capable of

discriminating cancer from normal mucosa with a PPV of 1 and a NPV above 0.5. Short methylation independent amplification was considered feasible for the five genes. Digital PCR (Methyl- BEAMing) assays were designed and their sensitivity and specificity were reassessed in silico using the probes located within the amplicon (see online supplementary file 4). The quantitative aspect of the assay was privileged over sensitivity, for better serving the purpose of monitoring tumour burden in advanced disease patients. Digital MIQE checklist can be found in online supplementary file 3.

Assessment of methylation markers in tissue from patients with CRC

Methylation status was evaluated in FFPE tissue DNA for the five markers defined above (*EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*). Amplification was successful in all cases. Significantly higher methylation levels were observed in tumour tissue compared with their normal counterpart ($p < 0.0001$), and remained high in an independent set of non-macro-dissected tumour specimens (figure 2). Average methylation (and range) for normal tissues was 0.6% (0–3), 7.9% (0–28), 0.5% (0–9), 0.3% (0–2) and 2.3% (0–12) for *EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*, respectively. Average methylation (and range) for matched tumour tissues was 42.2% (0–92), 67.1% (18–97), 51.7% (2–96), 43.5% (0–97) and 71.5% (31–97) for *EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*, respectively.

Detection of methylation markers in cfDNA of healthy individuals and patients with CRC

Plasma samples were obtained from a cohort of self-declared healthy individuals deliberately chosen above age 40 ($n=50$), and from a cohort of patients with mCRC ($n=182$). Methylation status was evaluated in a total of 364 cfDNA samples for the five markers (Figure 1 and online supplementary file 2G). Amplification was successful in all samples but one for *GRIA4* (0.3%), four cases for *ITGA4* (1.1%) and seven for *MSC* (1.9%) (online supplementary file 2G).

Marker prevalence in cfDNA

Considering only non-longitudinal mCRC samples from individual patients for prevalence purpose (figure 1), all markers showed significant differences in methylation distribution between self-declared healthy donors and patients with mCRC (U test: $p < 0.0001$, Figure 3A–E and online supplementary file 2G). Average methylation (and range) for self-declared healthy donors was 0.5% (0–7.8), 0.5% (0–17), 0.2% (0–2.2), 0% (0–0.2) and 0% (0–0.6) for *EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*, respectively. Average methylation (and range) for non-longitudinal mCRC samples was 26.2% (0–98.4), 33.5% (0–99.7), 33.2% (0–99), 26.3% (0–100) and 37.9% (0–100) for *EYA4*, *GRIA4*, *ITGA4*, *MAP3K14-AS1*, *MSC*, respectively. Receiver operating characteristic curve (ROC) analyses were performed for each marker in order to evaluate their performance and establish a clinically relevant positivity threshold to discriminate self-declared healthy donors from patients with mCRC (see online Supplementary file 7). Cut-off values were 0.7% for *EYA4*, 0.79% for *GRIA4*, 1.06% for *ITGA4*, 0.24% for *MAP3K14-AS1*, 0.9% for *MSC* and 0.68% when averaging the five markers. Only two plasma samples from healthy donors showed an average methylation above the threshold (Figure 3G). This was due to high methylation values in *GRIA4* and *EYA4* for one individual (aged 43 years), and in *EYA4* for the second (aged 62 years). One hundred and thirty-nine (76%) patients with mCRC displayed an average methylation above the threshold. Positivity, defined as the methylation value over cut-off thresholds and number of methylated events above the limit of blank (LOB), was observed in 71.4% for *EYA4*, 68.5% for *GRIA4*, 69.7% for *ITGA4*, 68.1% for *MAP3K14-AS1*, 65.1% for *MSC*, respectively. Considering that positivity in a single marker would be enough to track tumour burden, we evaluated that 156 cases (86%) showed positivity in at least one marker. When methylation values were positive, all markers were correlated (figure 3H and online Supplementary file 8). Total DNA amount assessed by genome equivalent per millilitre also significantly discriminated self-declared healthy donors from

patients with mCRC, although with lower specificity ([Figure 3F](#) and online Supplementary file 7).

Marker prevalence in tumour tissue and matched metachronous cfDNA samples

Tumour tissue DNA and matched metachronous cfDNA samples were available for a small subset of patients for which primary tumour had been resected (n=39). Marker negativity in cfDNA samples was not explained by absence of methylation in the matched tumour tissue counterpart (see online Supplementary file 9).

Association between cfDNA methylation and clinicopathological features

Using univariate analyses, age, treatment status and *BRAF* or *RAS* oncogenic mutations were not associated with different methylation values (see online Supplementary file 10; Supplementary file 2). High carcinoembryonic antigen (CEA) level (>5 ng/mL) showed a non-significant trend for higher methylation values in cfDNA (p=0.11). Male gender was significantly associated with lower methylation values (p=0.029), while the presence of unresected primary tumour (p=0.002), bulky disease (defined as massive tumour involvement of >50% of liver or lungs; p=0.012) or multiple metastatic lesions (p=0.023) were significantly associated with higher methylation values. The sum of target lesions as *per* Response Evaluation Criteria for Solid Tumours (RECIST)³⁵ from available CT scans was also associated with high methylation in the highest quartiles. Altogether these findings strongly suggest an association between release of methylated cfDNA and tumour burden.

Longitudinal assessment

Among the 182 patients with mCRC, 45 were followed-up longitudinally ([figure 1](#)); of those, seven were excluded due to absence of any positive marker at baseline (n=6) or inadequate follow-up (n=1). Methylation changes, between baseline and longitudinal plasma samples (obtained within 20 days from the first radiological evaluation) were annotated with the tumour response status to therapy (see online Supplementary file 11).

Significant lower methylation values were detected in samples collected close to radiological assessment of clinical benefit (defined as objective disease stabilisation or partial response as per RECIST criteria) for all markers but *EYA4*. In comparison, samples collected close to documented objective tumour progression showed a non-significant trend towards increased methylation values for all markers. This suggests that cfDNA methylation changes could be associated with tumour burden dynamics. Therefore, we investigated whether longitudinal follow-up of methylation could track tumour burden over time. For this purpose, for each longitudinal time-point an average of selected markers (ASM) was calculated based exclusively on the loci which displayed positive methylation in the baseline.

Monitoring of mCRC response to conventional chemotherapy

Ten cases received conventional chemotherapy regimens (FOLFOX, FOLFIRI, with or without bevacizumab). Two cases were excluded because they were negative at baseline. All patients carried *KRAS* or *BRAF* mutated tumours (considered as early events in tumourigenesis) allowing correlative assessment with methylation (figure 4). ASM was used for longitudinal monitoring. For most time-points, ASM dynamics recapitulated tumour burden changes as assessed by imaging, with a decrease preceding partial response or stable disease, while an increase or stable ASM anticipated progression. For all cases with known mutations in the corresponding tissue, *KRAS* or *BRAF* mutant levels in cfDNA paralleled the ASM. At a few time-points negative for mutation detection and displaying low DNA amount, ASM was around 1%, which could reflect either higher sensitivity of multiple methylated marker testing or possible overestimation of the current assays (see online Supplementary file 12).

Monitoring of mCRC response to targeted therapy

Additional six cases with longitudinal follow-up were treated with the EGFR targeted antibody panitumumab based on *RAS* wild-type status (figure 1). One case displaying a

baseline sample negative for methylation was excluded. Four out of five cases (figure 5) demonstrated emergence of a resistance causative alteration at progression which could be retrospectively analysed over time. In three individuals, progression was associated with the emergence of *KRAS* alterations (figure 5A-C),⁴ in one by *MET* gene amplification³² (figure 5D). In all cases, ASM increased in parallel with the emergence of the resistance causative alteration. However, ASM values in plasma were much more abundant than the percentage of mutant *KRAS* alleles in two cases. In two patients, panitumumab was followed at progression by standard chemotherapy (irinotecan) that was associated with decrease of *KRAS* cfDNA level independently of the methylation dynamics. The remaining case for which the molecular mechanism of resistance to EGFR target therapy remained unexplained could still be monitored in cfDNA with an increase in the ASM value at progression (figure 5E).

Application of methylated circulating DNA monitoring in a clinical trial with temozolomide in chemorefractory mCRC with MGMT hypermethylation

We investigated the use of methylated cfDNA in 29 cases from the TEMECT trial (EudraCT number 2012-003338-17), which assessed efficacy of temozolomide treatment in patients with chemorefractory mCRC selected based on their O-6-methylguanine- DNA methyltransferase (MGMT) methylated status in the primary tumour tissue.³⁴ Three cases without any positive methylated marker at baseline and one case without longitudinal collection were excluded (figure 1). To explore whether methylated markers in liquid biopsy could be used as a surrogate to imaging, we considered the best methylation change over time, similarly to what is usually performed with imaging-based RECIST criteria. To this aim, the ASM at a longitudinal timepoint was subtracted from the ASM value at baseline, and the best methylation change (lowest) over the course of treatment was selected for correlative assessment with radiological response (see online supplementary file 2I). A decrease in methylation was associated with clinical benefit as

evaluated by RECIST (stable disease or partial response; [figure 6A](#), PPV=0.82; NPV=0.79, $p=0.0048$). Considering the best ASM change over time, a decrease in ASM was associated with improved progression-free survival ($p=0.039$, HRrelapse=0.48 (0.17–0.87); [figure 6B](#)).

Discussion

The current use of imaging for follow-up in CRC suffers from limitations potentially leading to overtreatment, delays in treatment reorientation and potential side effects of exposure to imaging contrast agents. The evaluation of serum protein levels such as CEA offers a rapid and cost-effective way to measure disease evolution, but is impaired by limited sensitivity and specificity, the latter especially during treatment courses due to inflammation and release of protein in the bloodstream.³⁶ Furthermore, a fraction of patients with mCRC does not show detectable plasmatic CEA levels during the natural history of the disease.³⁷ Longitudinal evaluation of cancer-specific mutations in cfDNA has been exploited to identify relapse after surgery⁵ or during treatment with both standard chemotherapies and targeted agents,^{1 4} and it demonstrated great specificity and sensitivity. However, these studies must rely on either mutational hotspots with partial prevalence (only up to 50% considering all alterations of *KRAS* and *BRAF*) or on personalised assay design after identification of a variant through massive parallel sequencing.^{4 5} Alterations in methylation patterns present an advantage, as they are limited to specific regions of the genome, allowing for a universal assay design compatible for population studies. Moreover, their prevalence is usually high, which triggered their implementation as early diagnostic assays.³⁸ We confirmed the proof-of-concept work from Garrigou *et al*/³⁹ that DNA methylation in cfDNA may be employed to track response during therapy in mCRC, enabling non-invasive monitoring of tumour burden. To go beyond previous efforts in this area, we decided to perform a marker discovery analysis

using CRC cell lines. This strategy allowed removal of background signal coming from stroma, which has recently been described to impair cancer signal specificity in genome-wide analyses.⁴⁰ Further validation in independent cohorts (in silico) or in tissue samples confirmed that these markers were cancer specific and not a consequence of cell line establishment. To our knowledge, only two studies from the same group evaluated methylation markers dynamics in mCRC cases on treatment.^{39 41} In their manuscript, Garrigou *et al* detected methylation of *WIF1* and *NPY* by liquid biopsy in 80% mCRC, and evaluated their levels over time in cfDNA from three mCRC cases under chemotherapy treatment.³⁹ In a follow-up study, combining *WIF1* and *NPY* methylation Garlan *et al* showed 69.2% positivity in KRAS/BRAF/TP53 wild-type mCRC.⁴¹ While these markers were identified by Roperch *et al*²¹ using the Illumina GoldenGate methylation arrays (lower coverage than the Illumina Infinium), they were sorted out from our pipeline. *NPY* was discarded due to positivity in normal mucosa and in whole blood samples which contain white blood cells as the main contaminant of cfDNA. *WIF1* instead did not satisfy our stringent threshold criteria due to limited differential methylation between normal healthy/normal adjacent mucosa and tumour. Methylation of *WIF1* in normal mucosa was previously described in a small dataset⁴² warranting further investigation to verify the specificity of this marker. Among other established liquid biopsy markers for CRC, *SEPT9* was also filtered out from the analysis due to distance between the significantly differentially methylated probes above the 150 bp threshold. Among the methylated loci identified in our work, *EYA4* and *ITGA4* are known or putative tumour suppressor genes,^{43–46} while the functional role of the other markers -*GRIA4*, *MSC* and *MAP3K14-AS1*- in carcinogenesis remains to be elucidated; however, their in silico validation in independent cohorts as well as in tissue demonstrated their reliability in identifying tumour cells. Comparing cfDNA samples from self-declared healthy donors to patients with mCRC, all markers showed specificity above 0.85. The less efficient marker was *EYA4*, which

demonstrated low methylated values in all donor samples. Since the signal was higher than the LOB of the assay, we hypothesised that this could be attributed to age-related methylation in normal tissue. When evaluating plasma samples of patients with cancer, we established positivity as methylation value above a ROC threshold (allowing the best discrimination between healthy individuals and patients with cancer) and signal with positive events above the LOB. While these detection thresholds efficiently define the marker(s) to be followed over time, these assays are still suboptimal for other purposes such as early detection or minimal residual disease assesment. Despite heterogeneity among patients, the high prevalence of at least one methylated marker warrants including these loci in a panel as a blood test for detection of plasma DNA of tumour origin in patients with stage IV CRC. In our setting, all cases with intact primary CRC in situ displayed at least one positive methylation marker, confirming that cases in which primary lesions were not resected could be more efficiently tracked through liquid biopsy. As seen for *SEPT9*,⁴⁷ there was a positive correlation between cfDNA concentrations—here measured by genome equivalent per millilitre of plasma—and average methylation. However, in some samples with low total DNA content (below 20 000 genome equivalent), high methylation values were recorded; on the other hand, a subset of samples with high genome equivalent did not display detectable methylation in the selected loci. We hypothesise that these discrepancies could be explained by haemolysis during sample preparation and consequent contamination of plasma by leucocyte DNA, or by release of DNA from non-cancerous tissues during treatment, possibly due to inflammation or hepatotoxicity. Individual markers had prevalence around 65%, and only combining the positivity of all five methylated loci resulted in 86% overall prevalence, which is slightly higher but similar to what previously reported when both *NPY* and *WIF1* were assessed in the metastatic setting.³⁹ Yet, there were still 14% of mCRC cfDNA samples with undetectable circulating DNA methylation, which warrants further studies investigating

possible biological or technical bases. None of the recorded clinicopathological features correlated with lack of detectable methylation levels in cfDNA. Indeed, in our limited subset of matched tissue and plasma samples, marker negativity in cfDNA was not related to low methylation value in tumour tissue DNA. Consequently, we speculate that this discordance between sample types could be attributed to spatial or temporal intratumour epigenetic heterogeneity, [48](#) limited DNA shedding into circulation or suboptimal plasma specimen handling. Since tissue and microarray data displayed very low rate of non-methylated template (0% in our tissue dataset; 1.1% in microarray data), we expect that technical improvements at the detection level as well as at the DNA isolation level (eg, using higher plasma volume), will be required to achieve full penetrance of the assay. In addition to *NPY* and *WIF1*, other previously identified methylated loci in genes such as *SEPT9*,[16](#) [49](#) *VIM*[19](#) or *C9ORF50*[18](#) are amenable to liquid biopsy analyses, and their use in combination with our panel might also improve the positivity of cfDNA detection. One self-declared healthy donor displayed positivity in two markers and high GE content, possibly suggesting either a false-positive result or that this individual had an asymptomatic neoplastic lesion. However, the sample collection process (ie, de-identification) prevented us from verifying this hypothesis. It should be acknowledged that our digital PCR-based approach was not designed as a cancer diagnostic test, but rather optimised for its linearity and quantification ability of methylated DNA in advanced disease. Nevertheless, future studies are warranted to establish the methylation status and prevalence of the markers identified by our study in the earlier stages of colorectal neoplastic disease. This knowledge together with the development of assays that would privilege sensitivity over quantification are key to establish whether these findings could be relevant also in the setting of early detection. There was good correlation (above 0.55) between levels of circulating DNA methylation and early genetic events in colorectal tumourigenesis such as *KRAS* or *BRAF* mutations, which validates the possibility to use methylation without prior

knowledge of the tumour genetic pattern, as previously shown.^{39 41} Of note, four samples involved in the study were also assessed by massive parallel sequencing (data not shown) and presented genetic mutations at an allelic frequency comparable to the percentage of methylated markers, which highlights a possible role of our panel for plasma quality assessment prior to sequencing. In fact, massive parallel sequencing sometimes fails to detect tumour-specific somatic mutations due to low tumour content in cfDNA and methylation assay could therefore be used for checking tumour DNA enrichment in plasma as a quality control step. Methylation markers in cfDNA of patients treated with EGFR inhibitors, paralleled the emergence of resistance causative genetic alterations. This finding suggests the possibility to use these methylation markers as whole tumour DNA content normaliser in plasma. This will be of particular importance when qualitative assessment (presence or absence) of individual mutation variants in the blood is not enough to predict response. Interestingly, in two cases, the likely resistance mechanism (*KRAS* alteration) showed very low mutant allelic frequency in comparison to the estimated amount of tumour derived cfDNA (as judged by ASM). We hypothesised that either the tumour harboured additional unknown mechanisms of resistance or a small fraction of *KRAS* altered cells was enough to protect the main bulk of the neoplastic lesion via paracrine effectors as previously demonstrated.⁵⁰ In two cases that progressed through emergence of a *KRAS* mutation, levels of mutant alleles decreased on treatment with irinotecan, independently of methylation, suggesting that the fitness of *KRAS* mutant clones is dependent on the presence of anti-EGFR antibodies, as previously proposed.⁴ To our knowledge, very few studies evaluated longitudinally methylated cfDNA,^{39 41 51} and most works essentially focused on analysis of pretreatment samples to find predictive markers of response. Here, we showed that evolution of the methylation abundance over time demonstrated good prediction of response status during treatment with conventional therapies for mCRC. This suggests that longitudinal assessment of methylation could be

used in between radiological assessments for more accurate follow-up of the disease. We were also able to retrospectively assess a batch of samples that were collected in a clinical trial with temozolomide for the treatment of chemorefractory mCRC. Samples were not collected with the aim to directly compare cfDNA with CT scans. Imaging was not usually performed at the very same time-points when blood was drawn. Despite this limitation and the low response rate of mCRC on temozolomide treatment, dynamics of methylation could predict clinical benefit. This implicates that the monitoring of methylated circulating DNA might be used as a surrogate to imaging in order to evaluate treatment efficacy and might help reducing delays in therapeutic reorientation. In fact, with the emergence of concepts such as early tumour shrinkage associated with long-term outcome,^{52 53} short-term evaluation of pharmacodynamic response using liquid biopsy might become common practice. In summary, we presented here a panel of five methylated universal markers of circulating DNA of tumour origin that can be efficiently used to monitor advanced CRC on most currently available treatments. We hypothesise that combining radiological and repetitive cfDNA assessments will improve monitoring of patients with mCRC and would help clinicians to adjust treatment more efficiently by adopting more timely surgical intervention or early therapeutic reorientation.

Acknowledgements

The authors are grateful to the members of the Core Facilities (Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Italy) for their wonderful support and dedication. The authors thank the different members of the lab for their invaluable comments and discussion, in particular Beth Van Emburgh for editing the manuscript and Pamela Arcella for her technical support. The authors thank the study subjects from clinical trials (DETECT-01: EudraCT number 2011-002080-21; TEMECT: EudraCT number 2012-003338-17) involved in the study for providing some of the samples used in this work. The authors thank the

study subjects and staff of the University of Washington Medical School's GICaRes biorepository and the Fred Hutchinson Cancer Research Center's ColoCARE study for providing some of the samples used in this study. The authors thank the Fred Hutchinson Cancer Research Center's genomics shared resource for generating some of the methylation array data in this study. The authors thank Béla Molnár for critical feedback. The authors thank Silvia Marsoni from Candiolo Cancer Institute-FPO, IRCCS, for fruitful discussions. The authors also thank Simona De Stefanis for secretarial support. The results presented here are in part based on data generated by the TCGA Research Network.

Contributors

LB, AB, ASB and FDN participated in the design of the study. AA, AP, AC, ABi, CCr, SB, KB, PR, KBM, SS and ASB contributed to the collection and retrieval of clinical data of blood samples. AA, AC, AV, MT, SS and ASB contributed to the collection and retrieval of tissue samples. LB, AP, CF, CCr, AC, BM, GS and DO contributed to blood sample preparation and processing. LB, SMO, SMa, SG, GM, WG and ME contributed to the genome-wide methylation experiments. PZ contributed to establishing the pipeline for in silico and wet validation of GRIA4. LB performed the bioinformatics analyses of the genome-wide methylation experiments. LB, CF and DO performed methylation analyses in cfDNA samples. BM and GS performed genetic alteration analyses in cfDNA samples. LB, BM and GS interpreted the results in cfDNA. LB and FDN wrote the manuscript. All authors critically reviewed and commented the manuscript.

Funding

Research in the authors' laboratory was supported by grants AIRC IG n. 17707 (FDN); AIRC IG n. 16788 (AB); Fondo per la Ricerca Locale (ex 60%), Università di Torino, 2014

and 2017 (FDN); grant Fondazione Piemontese per la Ricerca sul Cancro—ONLUS Farmacogenomica 5 per mille 2009 MIUR (FDN), grant Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 per mille 2011 Ministero della Salute (AB and FDN); European Community's Seventh Framework Programme under grant agreement no. 602901 MErCuRIC (AB and FDN); H2020 grant agreement no. 635342-2 MoTriColor (AB); IMI contract n. 115749 CANCERID (AB); Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 per mille 2014 Ministero della Salute (AB). Investigators at Niguarda Cancer Centers are supported by the following grants: Terapia Molecolare dei Tumori (AS-B and SS) and Dynamic of Tumor Evolution & Therapy (AS-B) from Fondazione Oncologia Niguarda Onlus; Associazione Italiana per la Ricerca sul Cancro (AIRC) 2010 Special Program Molecular Clinical Oncology 5x1000, project 9970 (SS and AB). PZ is partly funded by Fondazione Banco di Sardegna (2012) and Regione Autonoma della Sardegna (CRP-79303). Partly supported also by NIH grants 1R01CA194663, 1R01CA189184, U01CA152756 and P30CA15704 to WMG. Ludovic Barault was the recipient of a postdoctoral fellowship from Fondazione Umberto Veronesi in 2013 and 2015, and of 'Assegno di Ricerca' from the University of Torino in 2016.

Competing interests

AB reports personal fees (scientific advisory board member) from Horizon Discovery, personal fees (scientific advisory board member) from Biocartis, personal fees (Consultant) from Novartis, personal fees (Consultant) from Roche, personal fees (Consultant) from Illumina. AB and FDN reports grants from Trovogene, outside the submitted work. In addition, FDN and PZ have a patent 102017000072650 pending. All the other authors have nothing to disclose.

Ethics approval

Niguarda Cancer Center ethics committee; San Giovanni Battista Hospital ethics committee.

Provenance and peer review

Not commissioned; externally peer reviewed.

Author note

AS-B and FDN contributed equally as co-senior authors.

References

- 1 Bettgowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early and late-stage human malignancies. [Sci Transl Med](#) 2014;6:ra24.
- 2 Crowley E, Di Nicolantonio F, Loupakis F, et al. Liquid biopsy: monitoring cancer genetics in the blood. [Nat Rev Clin Oncol](#) 2013;10:472–84.
- 3 Berger AW, Schwerdel D, Costa IG, et al. Detection of hot-spot mutations in circulating cell-free DNA from patients with intraductal papillary mucinous neoplasms of the pancreas. [Gastroenterology](#) 2016;151:267–70.
- 4 Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. [Nat Med](#) 2015;21:827.
- 5 Tie J, Wang Y, Tomasetti C, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. [Sci Transl Med](#) 2016;8:346ra92.
- 6 Reinert T, Scholer LV, Thomsen R, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. [Gut](#) 2016;65:625–34.
- 7 Berger AW, Schwerdel D, Welz H, et al. Treatment monitoring in metastatic colorectal cancer patients by quantification and KRAS genotyping of circulating cell-free DNA. [PLoS One](#) 2017;12:e0174308.

- 8 Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. [Nat Med](#) 2008;14:985–90.
- 9 Spindler KL, Pallisgaard N, Appelt AL, et al. Clinical utility of KRAS status in circulating plasma DNA compared to archival tumour tissue from patients with metastatic colorectal cancer treated with anti-epidermal growth factor receptor therapy. [Eur J Cancer](#) 2015;51:2678–85.
- 10 Taly V, Pekin D, Benhaim L, et al. Multiplex picodroplet digital PCR to detect KRAS mutations in circulating DNA from the plasma of colorectal cancer patients. [Clin Chem](#) 2013;59:1722–31.
- 11 Lehmann-Werman R, Neiman D, Zemmour H, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. [Proc Natl Acad Sci U S A](#) 2016;113:E1826–E1834.
- 12 Hardy T, Zeybel M, Day CP, et al. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. [Gut](#) 2017;66:1321–8.
- 13 Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. [Nat Rev Cancer](#) 2011;11:726–34.
- 14 Chen WD, Han ZJ, Skoletsky J, et al. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. [J Natl Cancer Inst](#) 2005;97:1124–32.
- 15 Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. [N Engl J Med](#) 2014;370:1287–97.
- 16 Warren JD, Xiong W, Bunker AM, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. [BMC Med](#) 2011;9:133.
- 17 Church TR, Wandell M, Lofton-Day C, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. [Gut](#) 2014;63:317–25.
- 18 Lange CP, Campan M, Hinoue T, et al. Genome-scale discovery of DNA-methylation biomarkers for blood-based detection of colorectal cancer. [PLoS One](#) 2012;7:e50266.

- 19 Li M, Chen WD, Papadopoulos N, et al. Sensitive digital quantification of DNA methylation in clinical samples. [Nat Biotechnol](#) 2009;27:858–63.
- 20 Young GP, Pedersen SK, Mansfield S, et al. A cross-sectional study comparing a blood test for methylated BCAT1 and IKZF1 tumor-derived DNA with CEA for detection of recurrent colorectal cancer. [Cancer Med](#) 2016;5:2763–72.
- 21 Roperch JP, Incitti R, Forbin S, et al. Aberrant methylation of NPY, PENK, and WIF1 as a promising marker for blood-based diagnosis of colorectal cancer. [BMC Cancer](#) 2013;13:566.
- 22 Xue M, Lai SC, Xu ZP, Zp X, et al. Noninvasive DNA methylation biomarkers in colorectal cancer: A systematic review. [J Dig Dis](#) 2015;16:699–712.
- 23 Naumov VA, Generozov EV, Zaharjevskaya NB, et al. Genome-scale analysis of DNA methylation in colorectal cancer using infinium humanmethylation450 beadchips. [Epigenetics](#) 2013;8:921–34.
- 24 Luo Y, Wong CJ, Kaz AM, et al. Differences in DNA methylation signatures reveal multiple pathways of progression from adenoma to colorectal cancer. [Gastroenterology](#) 2014;147:418–29.
- 25 Barault L, Amatu A, Bleeker FE, et al. Digital PCR quantification of MGMT methylation refines prediction of clinical benefit from alkylating agents in glioblastoma and metastatic colorectal cancer. [Ann Oncol](#) 2015;26:1994–9.
- 26 Medico E, Russo M, Picco G, et al. The molecular landscape of colorectal cancer cell lines unveils clinically actionable kinase targets. [Nat Commun](#) 2015;6:7002.
- 27 Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. [Bioinformatics](#) 2014;30:1363–9.

- 28 Price ME, Cotton AM, Lam LL, et al. Additional annotation enhances potential for biologically-relevant analysis of the illumina infinium humanmethylation450 beadchip array. [Epigenetics Chromatin](#) 2013;6:4.
- 29 Chen YA, Lemire M, Choufani S, et al. Discovery of cross-reactive probes and polymorphic CpGs in the illumina infinium humanmethylation450 microarray. [Epigenetics](#) 2013;8:203–9.
- 30 Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. [Nat Rev Cancer](#) 2011;11:426–37.
- 31 Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. [BMC Bioinformatics](#) 2011;12:77.
- 32 Bardelli A, Corso S, Bertotti A, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. [Cancer Discov](#) 2013;3:658–73.
- 33 Amatu A, Sartore-Bianchi A, Moutinho C, et al. Promoter CpG island hypermethylation of the DNA repair enzyme MGMT predicts clinical response to dacarbazine in a phase II study for metastatic colorectal cancer. [Clin Cancer Res](#) 2013;19:2265–72.
- 34 Amatu A, Barault L, Moutinho C, et al. Tumor MGMT promoter hypermethylation changes over time limit temozolomide efficacy in a phase II trial for metastatic colorectal cancer. [Ann Oncol](#) 2016;27:1062–7.
- 35 Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). [Eur J Cancer](#) 2009;45:228–47.
- 36 Lech G, Słotwiński R, Słodkowski M, et al. Colorectal cancer tumour markers and biomarkers: recent therapeutic advances. [World J Gastroenterol](#) 2016;22:1745–55.
- 37 Nicholson BD, Shinkins B, Pathiraja I, et al. Blood CEA levels for detecting recurrent colorectal cancer. [Cochrane Database Syst Rev](#) 2015;12:CD011134.
- 38 Laird PW. The power and the promise of DNA methylation markers. [Nat Rev Cancer](#) 2003;3:253–66.

- 39 Garrigou S, Perkins G, Garlan F, et al. A study of hypermethylated circulating tumor DNA as a universal colorectal cancer biomarker. [Clin Chem](#) 2016;62:1129–39.
- 40 Isella C, Terrasi A, Bellomo SE, et al. Stromal contribution to the colorectal cancer transcriptome. [Nat Genet](#) 2015;47:312–9.
- 41 Garlan F, Laurent-Puig P, Sefrioui D, et al. Early Evaluation of Circulating Tumor DNA as Marker of Therapeutic Efficacy in Metastatic Colorectal Cancer Patients (PLACOL Study). [Clin Cancer Res](#) 2017;23:5416–25.
- 42 Patai ÁV, Valcz G, Hollósi P, et al. Comprehensive DNA methylation analysis reveals a common ten-gene methylation signature in colorectal adenomas and carcinomas. [PLoS One](#) 2015;10:e0133836.
- 43 De Carvalho DD, Sharma S, You JS, et al. DNA methylation screening identifies driver epigenetic events of cancer cell survival. [Cancer Cell](#) 2012;21:655–67.
- 44 Kim SJ, Tae CH, Hong SN, et al. EYA4 Acts as a new tumor suppressor gene in colorectal cancer. [Mol Carcinog](#) 2015;54:1748–57.
- 45 Ausch C, Kim YH, Tsuchiya KD, et al. Comparative analysis of PCR-based biomarker assay methods for colorectal polyp detection from fecal DNA. [Clin Chem](#) 2009;55:1559–63.
- 46 Chang E, Park DI, Kim YJ, et al. Detection of colorectal neoplasm using promoter methylation of ITGA4, SFRP2, and p16 in stool samples: a preliminary report in Korean patients. [Hepatogastroenterology](#) 2010;57:720–7.
- 47 Tóth K, Wasserkort R, Sipos F, et al. Detection of methylated septin 9 in tissue and plasma of colorectal patients with neoplasia and the relationship to the amount of circulating cell-free DNA. [PLoS One](#) 2014;9:e115415.
- 48 Martínez-Cardús A, Moran S, Musulen E, et al. Epigenetic homogeneity within colorectal tumors predicts shorter relapse-free and overall survival times for patients with locoregional cancer. [Gastroenterology](#) 2016;151:961–72.

- 49 Wasserkort R, Kalmar A, Valcz G, et al. Aberrant septin 9 DNA methylation in colorectal cancer is restricted to a single CpG island. [BMC Cancer](#) 2013;13:398.
- 50 Hobor S, Van Emburgh BO, Crowley E, et al. TGF α and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. [Clin Cancer Res](#) 2014;20:6429–38.
- 51 Fackler MJ, Lopez Bujanda Z, Umbricht C, et al. Novel methylated biomarkers and a robust assay to detect circulating tumor DNA in metastatic breast cancer. [Cancer Res](#) 2014;74:2160–70.
- 52 Cremolini C, Loupakis F, Antoniotti C, et al. Early tumor shrinkage and depth of response predict long-term outcome in metastatic colorectal cancer patients treated with first-line chemotherapy plus bevacizumab: results from phase III TRIBE trial by the Gruppo Oncologico del Nord Ovest. [Ann Oncol](#) 2015;26:1188–94.
- 53 Douillard JY, Siena S, Peeters M, et al. Impact of early tumour shrinkage and resection on outcomes in patients with wild-type RAS metastatic colorectal cancer. [Eur J Cancer](#) 2015;51:1231–42.

Figures

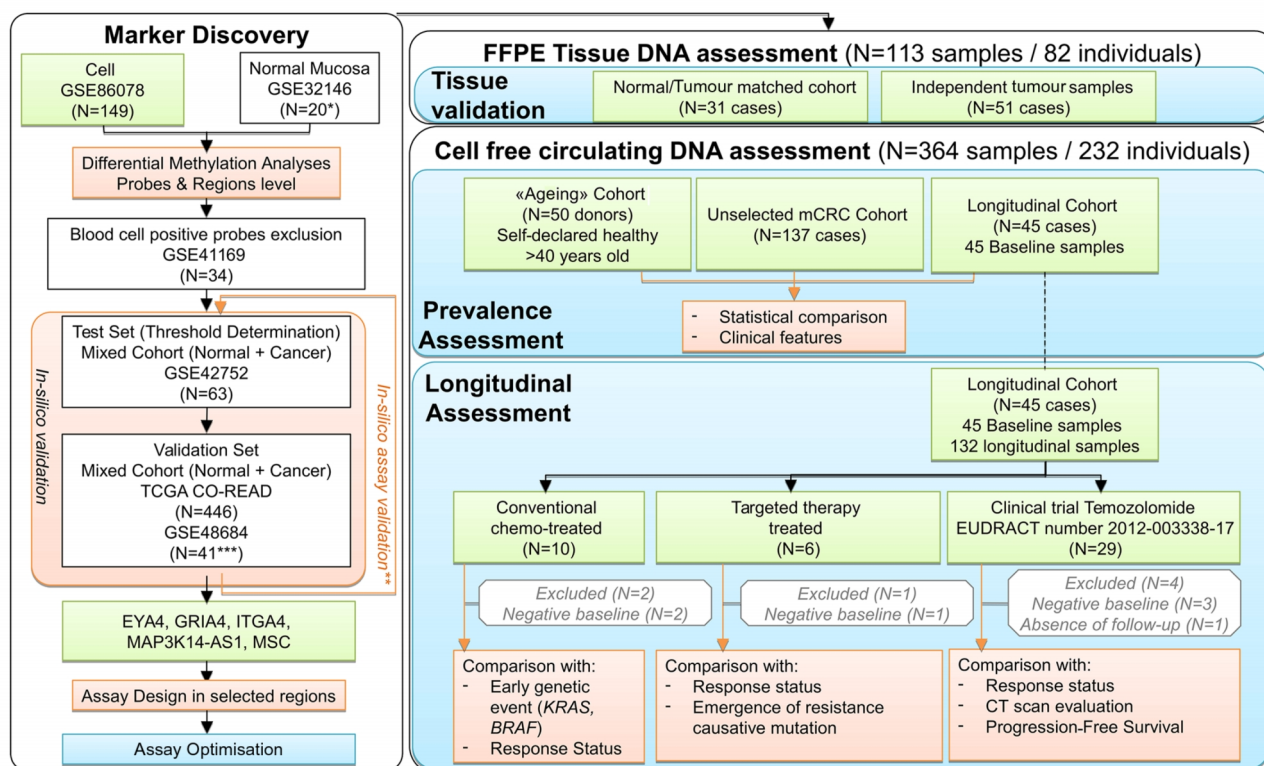


Figure 1 Workflow of the study. A multistep marker discovery analysis was first performed to identify highly prevalent cancer-specific markers. After design, assay probes were further validated in silico. Assays were optimised to achieve linear quantification over a wide methylation range (0.09%– 100%). Marker prevalence was first evaluated in tissue samples from 82 subjects with CRC. Then marker prevalence was evaluated in cfDNA in a total of 232 donors enrolled in the study among which 50 were de-identified self-declared healthy donors, and 182 patients with mCRC. Among mCRC cases, 45 were followed longitudinally and treated either with conventional chemotherapy, targeted therapy regimen or with temozolomide (TMZ) as part of a clinical trial. Methylation was analysed longitudinally for cases with positivity in at least one marker at baseline sample. Methylation dynamics was then compared with additional available clinical or molecular features. In green: unpublished data; in blue: bench experiments; in orange: bioinformatics or statistical analyses with clinical correlates; in grey: sample exclusion. *GSE32146 was used after removal of ulcerative colitis cases. **In silico validation was performed again

restricting the analysis to the probes included in the assay amplicon. ***Only normal healthy and peritumoural tissues were used from GSE48684.

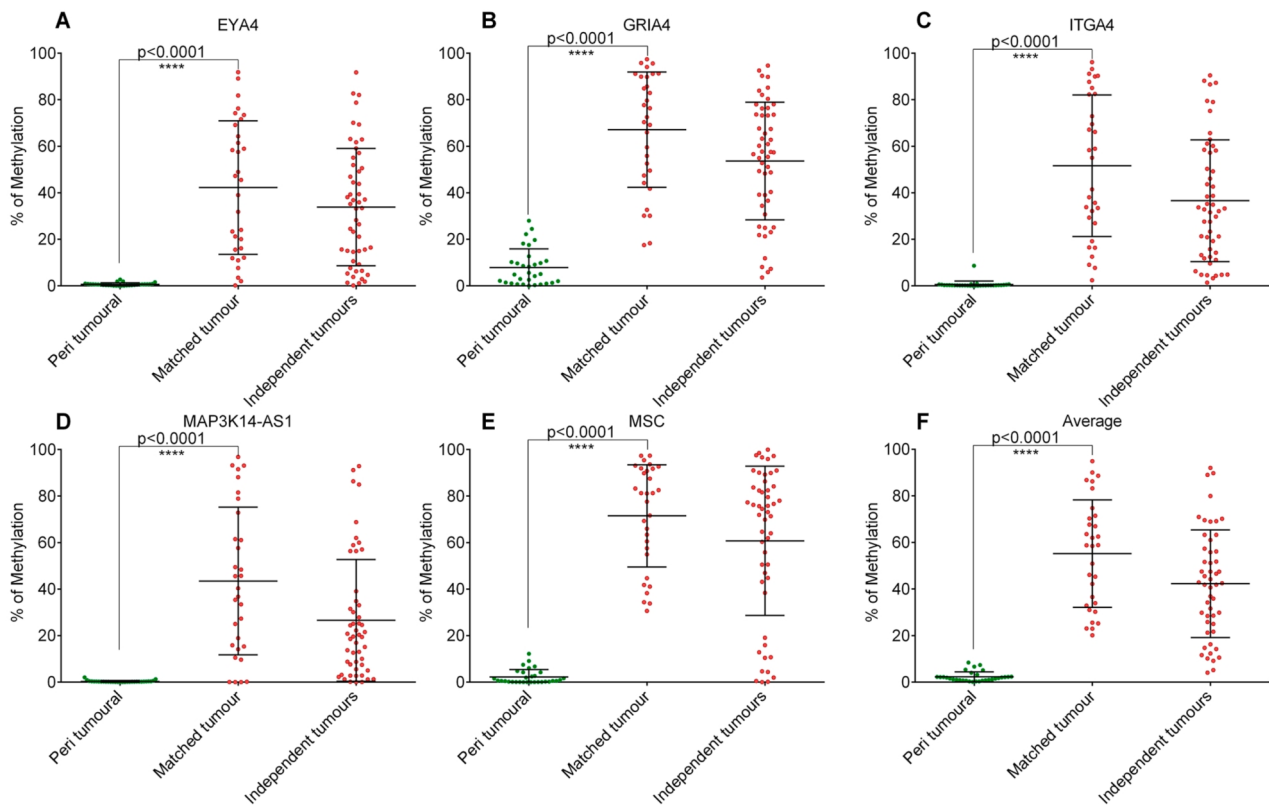


Figure 2 Prevalence of methylated markers in tissue DNA from patients with metastatic colorectal cancer (CRC). (A): *EYA4*, (B): *GRIA4*, (C): *ITGA4*, (D): *MAP3K14-AS1*, (E): *MSC*, (F): average of the five markers. Samples from 82 patients with CRC were analysed. A first set was composed of 31 cases from which tumour and peritumoural tissue DNA were available. A second set of independent 51 tumour tissue specimens was assembled from remaining DNA extracted during the enrolment of two clinical trials.

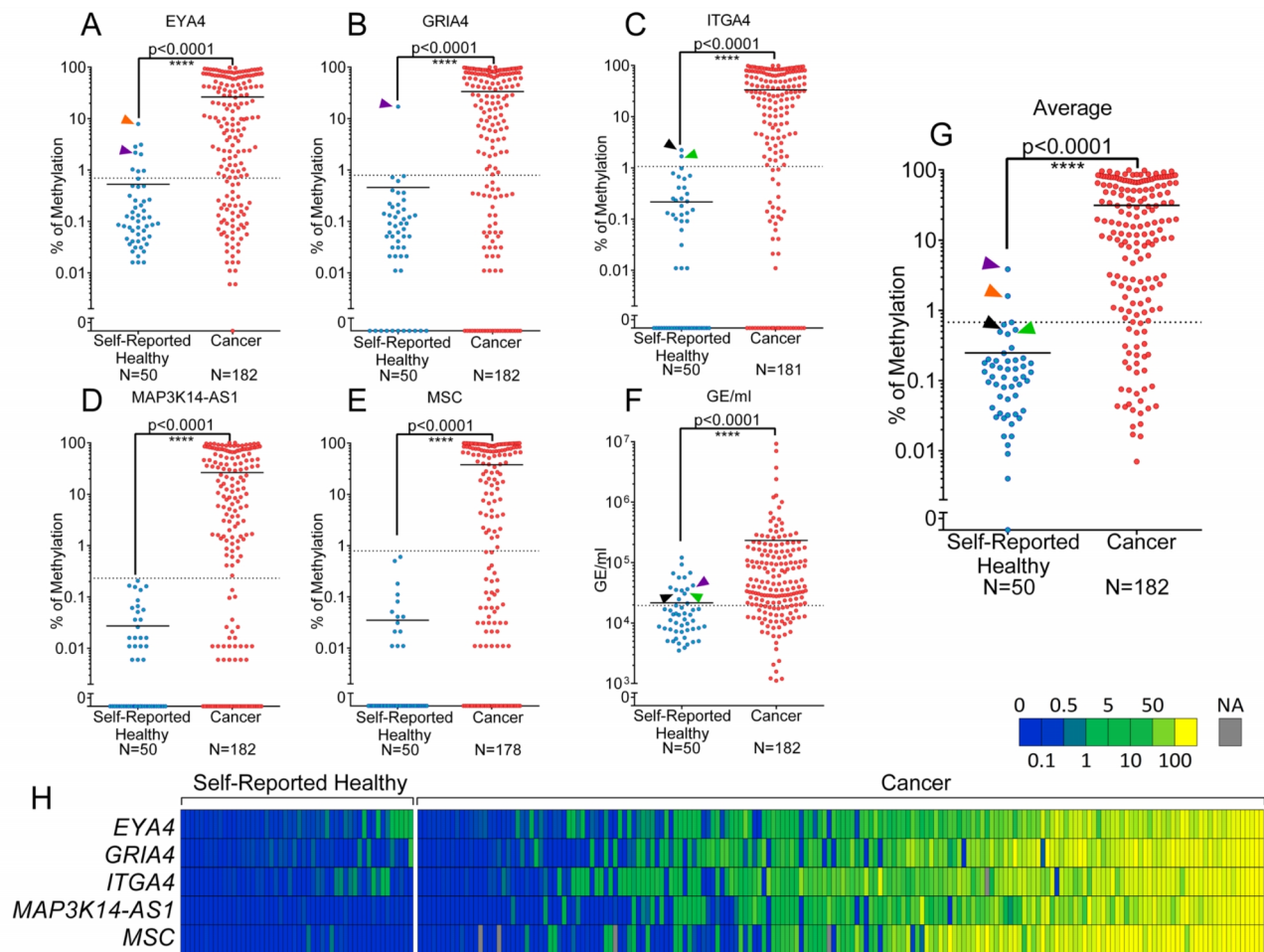


Figure 3 Prevalence of methylated markers in cell-free circulating DNA (cfDNA) and total amount of cfDNA. Plasma samples from 50 self-declared healthy donors (blue) and 182 patients with metastatic colorectal cancer (red) were analysed for the five selected markers. Group mean is represented by a horizontal bar. Mann-Whitney U test was performed to compare distribution in healthy patients and patients with cancer, which were all significantly different (with $p<0.0001$). Representation of individual markers: (A): *EYA4*, (B): *GRIA4*, (C): *ITGA4*, (D): *MAP3K14-AS1*, (E): *MSC*, (F): genome equivalent/mL (GE/mL). (G): Representation of average methylation signal. Self-declared healthy donor plasma samples presenting more than one outlying value were indicated by coloured arrows; the dashed line corresponds to the threshold established by receiver operating characteristic curve (ROC) analyses available in online Supplementary file 7. (H): Heatmap of methylation values sorted by average methylation.

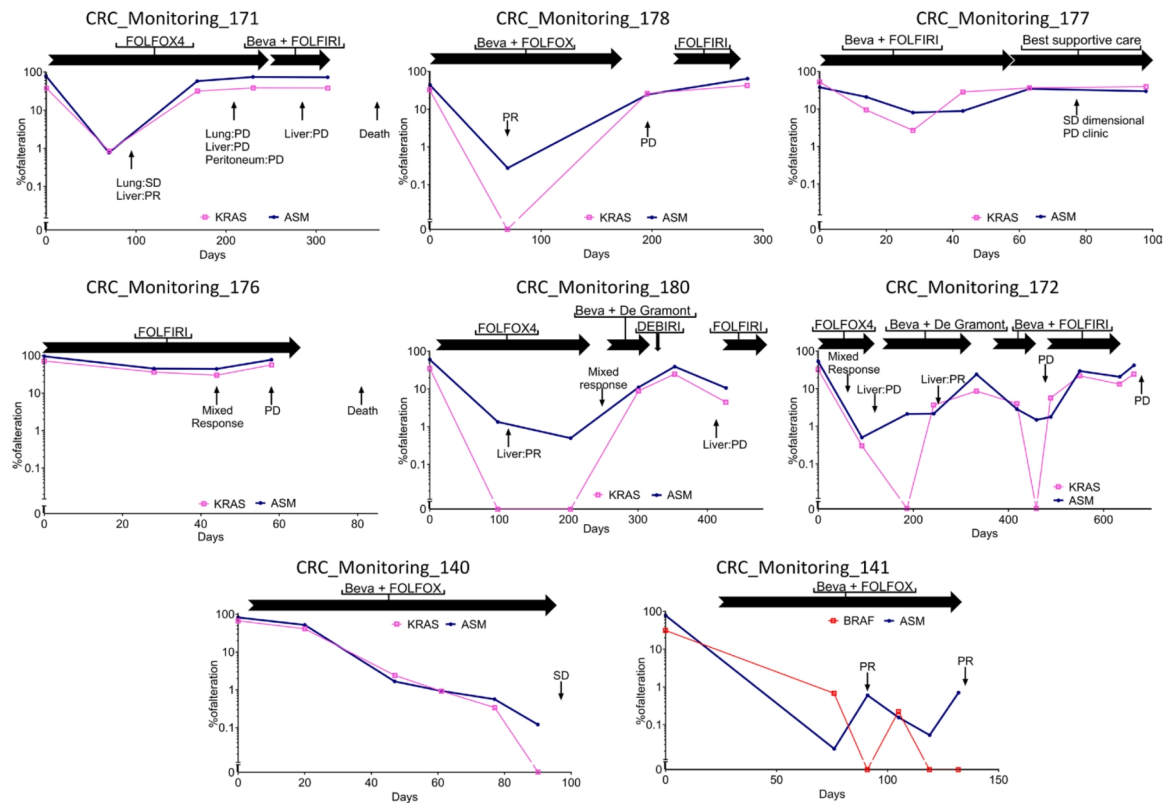


Figure 4 Average of selected markers (ASM) in cell-free circulating DNA dynamics in eight metastatic colorectal cancer (CRC) patients treated with conventional chemotherapy regimens. ASM is plotted in blue, while *KRAS* mutations are plotted in pink and *BRAF* in red. Methylation and genetic mutations evolve in parallel demonstrating the possibility to use methylation instead of genetic alterations for tracking response. Response status is indicated with arrows. PR, partial response; SD, stable disease; PD, progressive disease. Treatment periods are indicated as horizontal black arrows with corresponding chemotherapy regimens.

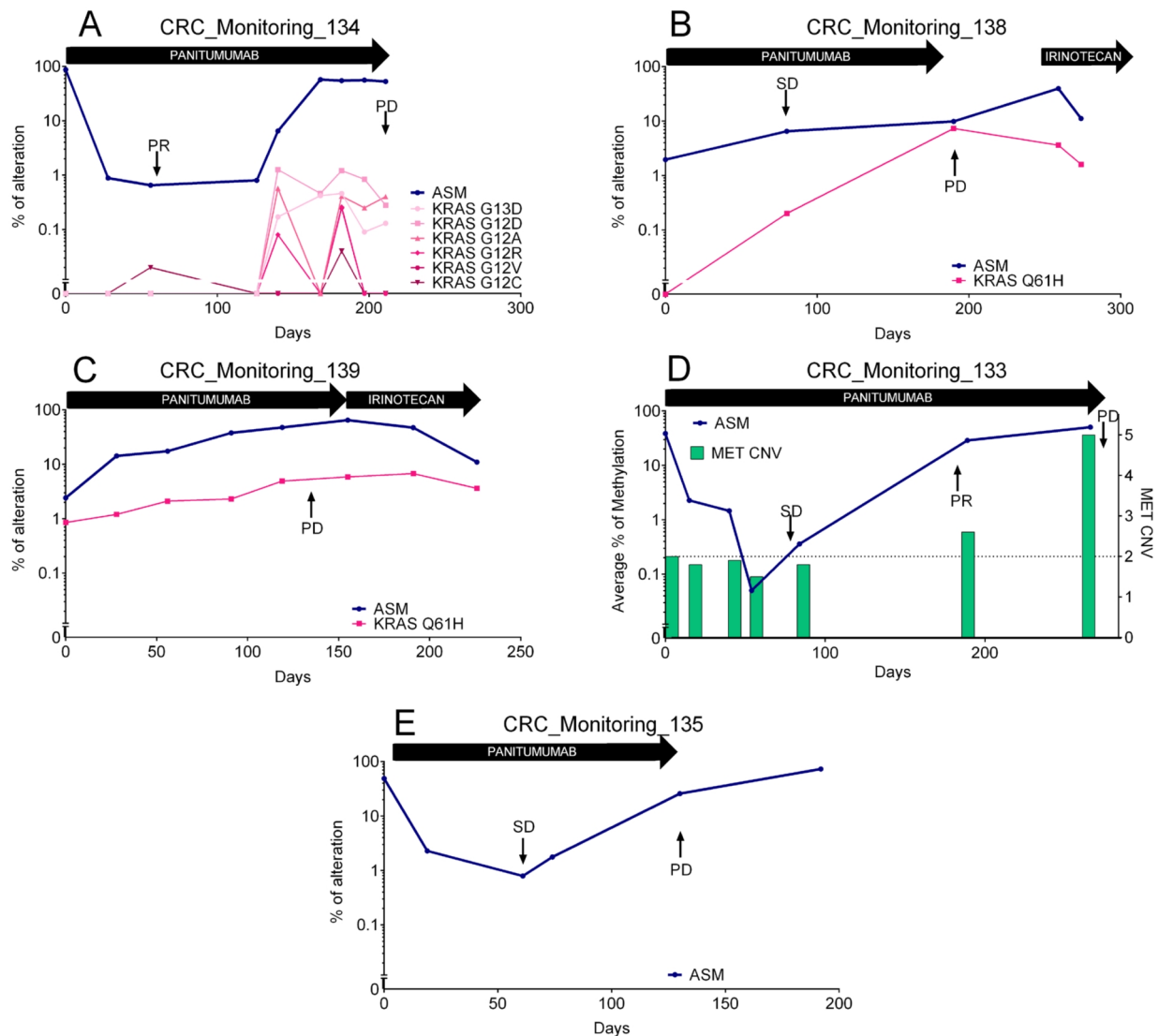


Figure 5 Average of selected markers (ASM) in cell-free circulating DNA dynamics in five metastatic colorectal cancer (CRC) patients treated with panitumumab for whom resistance causative mutations were discovered at progression and retrospectively assessed longitudinally. (A–C): Resistance was acquired through the emergence of a *KRAS* alteration; (D): resistance was acquired through the emergence of *MET* gene amplification. In each case, increase in ASM parallels the appearance of resistance alterations. (E): A case in which resistance mechanism remained unidentified but for which ASM could detect relapse.

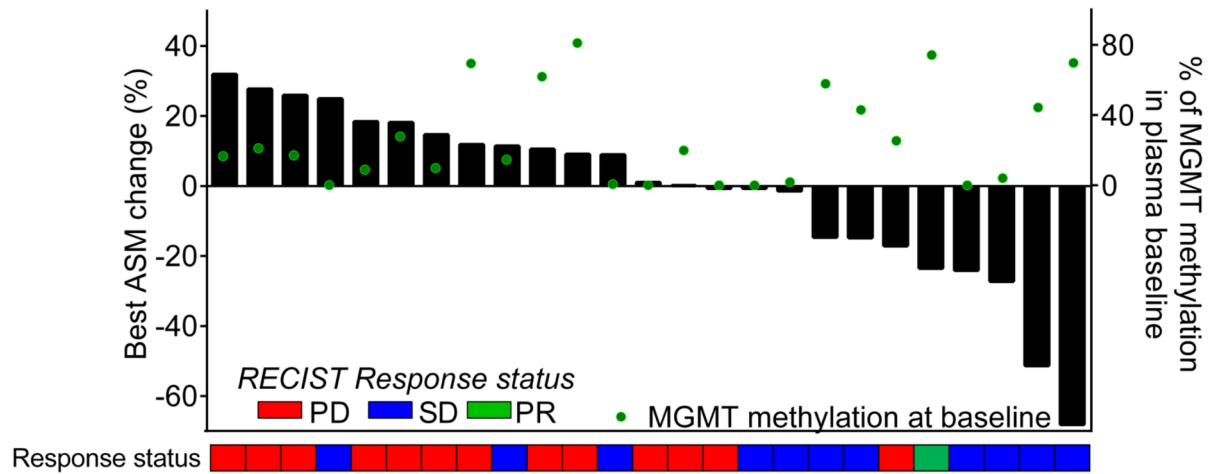
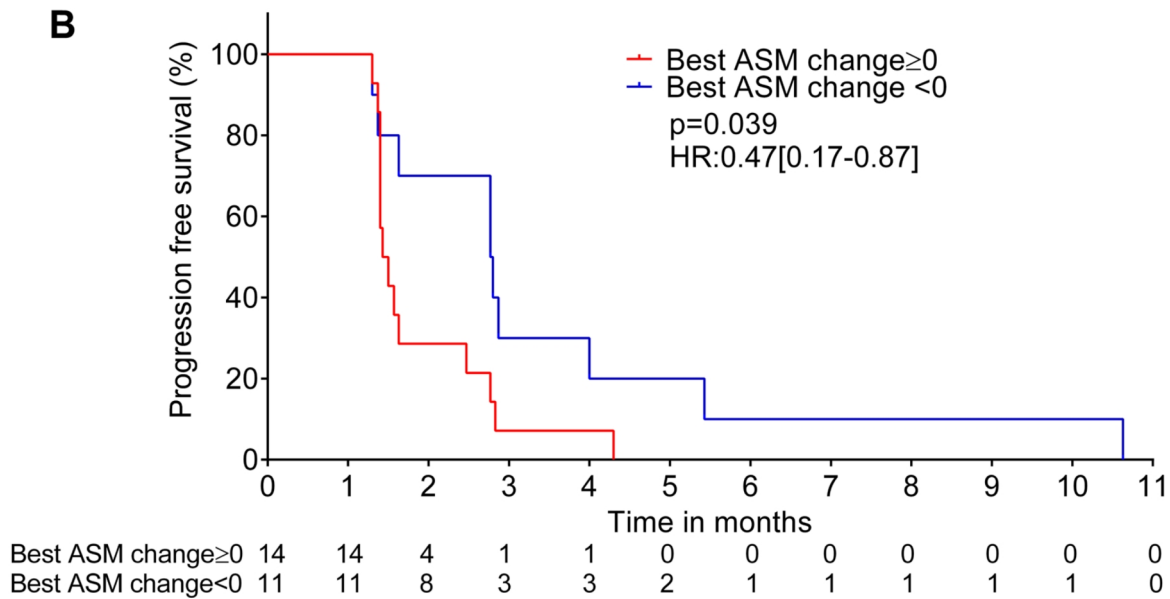
A**B**

Figure 6 Average of selected markers (ASM) in cell-free circulating DNA dynamics assessment in patients with metastatic colorectal cancer treated with temozolomide within a clinical trial. (A): Comparison of ASM changes to response status evaluated by RECIST. The best ASM changes were plotted as a waterfall plot. Response status of patients evaluated by RECIST is plotted as a heatmap. (B): Progression-free survival according to best ASM change. Negative ASM change is associated with improved PFS. HR was estimated by log rank approach.

Supplementary File 1 - Supplementary Material and Methods

Cell lines and genome wide DNA methylation data processing and retrieval

A collection of 149 cell lines of intestinal origin (**Supplemental File 2A**) was assembled from different worldwide cell line banks or academic laboratories, as described previously [1].

DNA samples were extracted by Wizard SV Genomic DNA Purification System (Promega), and cell identity was confirmed using the Gene Print 10 System (Promega). DNA samples were checked for integrity by 1% agarose gel, and quantity via Picogreen quantification. All samples were bisulfite converted using the EZ DNA methylation Gold kit (Zymo Research) with a minimum input of 500 ng of gDNA following the manufacturer's instructions. Infinium HumanMethylation450 BeadChip arrays were carried out according to the Illumina Infinium HD methylation protocol. Raw methylation profiles have been deposited on Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>; accession number: GSE86078).

GSE32146 was downloaded from GEO and considered as a control set (*i.e.* cancer unrelated normal mucosa, after removing the ulcerative colitis cases), for comparison with the cell lines. GSE41169 was downloaded from GEO and considered as a blood control (*i.e.* healthy donors) for verification of tissue specificity of the loci of interest.

GSE42752 [2] was downloaded from GEO and considered as a test cohort for the establishment of the beta-value threshold used for the *in-silico* validation. Level 1 data from the TCGA colorectal adenocarcinoma (COAD/READ) (<http://cancergenome.nih.gov/>; <https://gdc.cancer.gov/>) were downloaded and defined the validation cohort used for the *in-silico* validation. Further validation in normal tissues was performed GSE48684 [3]

Data Preprocessing and Marker Discovery Analysis

All raw data (IDAT files) were preprocessed in R Bioconductor using the minfi package [4] via the preprocessIllumina function (using background correction and control normalization) to achieve data normalized similarly to GEO downloaded datasets. The individual probe signal was removed when the detection p-value was above 0.05, and probes were removed from the dataset if more than 1% of the dataset contained no data. Our dataset was then merged with the other publically available cohorts before removal of probes containing SNPs [5], demonstrating sexual dimorphism [6] or located on sex chromosomes.

Differential probe analysis was performed using the lmFit function from limma package (choosing an adjusted p-value threshold of 1×10^{-35} and a minimum delta beta-value of 0.8). Probes were verified to not display any methylation in leukocytes (GSE41169; maximum beta-value allowed = 0.1) insuring absence of false positivity in blood tests due to the high methylation value of blood cells. Differential region analysis was performed using the bumpHunter function from the minfi package choosing a threshold of 0.8 and restricting the region to those represented by at least two probes ($L \geq 2$). Differentially methylated probes were limited to those overlapping differentially methylated regions, and “liquid biopsy” assessable loci were defined.

GSE42752 data were averaged for each of the genomic regions defined in the previous step according to sample type (normal and normal adjacent versus adenocarcinoma). Thresholds were calculated using Receiver operating characteristic analyses performed with the pROC package in R Bioconductor [7] considering normal and adjacent mucosa as positive outcome and cancer as negative; only loci showing a threshold below 0.35 were kept. Each characterized threshold were used to stratify the TCGA COREAD cohort, defining a positive predictive value (PPV) and negative predictive value (NPV) for discriminating normal adjacent from tumor tissue. Normal healthy and peri-tumoral

samples from GSE48684 [3] were also controlled for absence of methylation above the threshold.

Tissue collection and DNA isolation

Formalin fixed paraffin embedded tissues originated from two different cohorts. One containing macro-dissected tumor and normal adjacent tissues (N=31 cases; originating from several local hospitals) which were controlled for tumor purity and assembled at Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda (Milan, Italy) and from which DNA was newly extracted. A second cohort of independent tumor tissues (N=51) was assembled from remaining DNA extracted during the enrollement of the DETECT (EUDRACT 2011-002080-21) [33] or TEMECT (EUDRACT number 2012-003338-17) [34] trials.

Plasma Collection and DNA preparation

De-identified whole blood samples from healthy donors were purchased from the Brigham and Women's Hospital specimen bank (Boston, USA). Collection was performed in K2EDTA tubes between November 2009 and April 2010 from self-declared healthy donors aged 40 years and above who visited the hospital for an annual routine examination. The donors' sex and age was maintained in the datafile. Plasma was isolated after 10 minutes of centrifugation at 1600 RCF, and stored at -80°C.

One hundred and eighty two cases of mCRC were enrolled in the study. One hundred and thirty-seven cases were selected for plasma timepoints based on plasma sample availability at a time when patients were presenting radiological evidence of disease. The remaining 45 cases were treatment baselines, selected for availability of longitudinal

follow-up (additional total of 132 longitudinal samples). Summary of the clinico-pathological features of the two cohorts can be found in **Supplementary File 2D** (mCRC patients clinical features are presented in **Supplementary File 2E**). mCRC plasma samples were collected in K2EDTA tubes at Ospedale Niguarda Ca' Granda (Milan, Italy) between September 2009 and December 2015 or at Ospedale Molinette (Turin, Italy) between June 2013 to July 2015. Plasma was isolated in a two-step protocol (10 minutes at 1600 RCF, 10 minutes at 3000 RCF) and stored at -80°C until processing. The study was conducted according to Good Clinical Practices and was approved by the local ethics committee. One milliliter of plasma was processed for DNA extraction with the QIAamp Circulating Nucleic Acid Kit (Qiagen) or with the Maxwell® RSC ccfDNA Plasma Kit (Promega) following the manufacturer's protocol. Change in extraction kit (from Qiagen to Promega) was decided to improve the experimental throughput in a semi-automated fashion using the Maxwell® RSC ccfDNA Plasma Kit with no major change in extraction quality or yield. Twenty microliters of DNA were used for bisulfite conversion using the EZ DNA methylation Gold kit (Zymo Research), following the manufacturer's protocol, with final elution in 40 µl.

Methylation and genetic alteration assays for cfDNA evaluation

Selected loci were investigated for possible methylation independent amplification. Primers were designed using the PyroMark® Assay Design SW 2.0 (Qiagen) with custom settings. Primers and probes are available in **Supplemental Table 2C**. *In-silico* validation of the assays was performed using the same steps as previously described: threshold estimation in GSE42752 and stratification in TCGA COREAD, using solely the probes which were located within the amplicon **Supplementary File 5**. Optimization and evaluation of Methyl-BEAMing assays was performed as previously described [10], with the difference that beads were run on a BD C6 Accuri system (Becton-Dickinson). *In-silico* validation results display for the five markers of interest are available in **Supplementary File 4**.

Digital Miqe checklist including limit of detection, limit of blank, quantification ability (linearity and sensitivity) is available in **Supplementary File 3**.

Evaluation of genetic alterations (*KRAS*, *BRAF* mutations and *MET* gene copy number) in cfDNA was performed as previously described [11, 12]. Primers and probes are either commercially available (Biorad) or were custom designed and are listed in **Supplemental File 2C**.

References

- 1 Medico E, Russo M, Picco G, Cancelliere C, Valtorta E, Corti G, *et al*. The molecular landscape of colorectal cancer cell lines unveils clinically actionable kinase targets. *Nature communications* 2015;**6**:7002.
- 2 Naumov VA, Generozov EV, Zaharjevskaya NB, Matushkina DS, Larin AK, Chernyshov SV, *et al*. Genome-scale analysis of DNA methylation in colorectal cancer using Infinium HumanMethylation450 BeadChips. *Epigenetics* 2013;**8**:921-34.
- 3 Luo Y, Wong CJ, Kaz AM, Dzieciatkowski S, Carter KT, Morris SM, *et al*. Differences in DNA methylation signatures reveal multiple pathways of progression from adenoma to colorectal cancer. *Gastroenterology* 2014;**147**:418-29 e8.
- 4 Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, *et al*. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* 2014;**30**:1363-9.
- 5 Price ME, Cotton AM, Lam LL, Farre P, Emberly E, Brown CJ, *et al*. Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. *Epigenetics & chromatin* 2013;**6**:4.
- 6 Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, *et al*. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* 2013;**8**:203-9.
- 7 Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, *et al*. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC bioinformatics* 2011;**12**:77.
- 8 Amatu A, Sartore-Bianchi A, Moutinho C, Belotti A, Bencardino K, Chirico G, *et al*. Promoter CpG island hypermethylation of the DNA repair enzyme MGMT predicts clinical response to dacarbazine in a phase II study for metastatic colorectal cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2013;**19**:2265-72.
- 9 Amatu A, Barault L, Moutinho C, Cassingena A, Bencardino K, Ghezzi S, *et al*. Tumor MGMT promoter hypermethylation changes over time limit temozolomide efficacy in a phase II trial for metastatic colorectal cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2016.
- 10 Barault L, Amatu A, Bleeker FE, Moutinho C, Falcomata C, Fiano V, *et al*. Digital PCR quantification of MGMT methylation refines prediction of clinical benefit from alkylating agents in glioblastoma and metastatic colorectal cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2015;**26**:1994-9.
- 11 Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, *et al*. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer discovery* 2013;**3**:658-73.

12 Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, *et al.* Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nature medicine* 2015;**21**:827.

Supplementary File 2A

List of samples used in the marker discovery analysis

SAMPLES USED FOR THE MARKER DISCOVERY

Sample_Name	Tissue_type	Donor_type	Dataset	Sentrix_ID	Sentrix_Position	Official_name
C10	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R02C02	C10
C106	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455219	R03C02	C106
C125PM	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875059	R06C02	C125PM
C146	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999876007	R02C01	C146
C170	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455225	R05C01	C170
C32	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R06C01	C32
C70	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R06C02	C70
C75	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450147	R05C02	C75
C80	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455207	R01C01	C80
C84	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455254	R05C02	C84
C99	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R06C01	C99
CACO2	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R06C01	CACO-2
CaR1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R06C01	CAR-1
CCK81	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R02C01	CCK81
CL11	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R01C02	CL-11
CL14	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455235	R06C01	CL-14
CL34	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875081	R01C02	CL-34
CL40	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455254	R02C01	CL-40
Co115	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455235	R05C01	Co-115
COCM1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R04C02	COCM-1
COGA1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R01C01	COGA-1
COGA12	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R06C01	COGA-12
COGA2	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R02C02	COGA-2
COGA3	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R03C02	COGA-3
COGA5	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875059	R01C01	COGA-5
COGA5L	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455197	R01C02	COGA-5L
COGA8	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875129	R03C02	COGA-8
COLO201	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R04C01	COLO-201
COLO205	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R01C02	COLO-205
COLO320DM	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R06C02	COLO-320DM
COLO320HSR	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R02C02	COLO-320HSR
COLO60H	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875129	R06C01	COLO-60H
COLO678	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R01C01	COLO-678
COLO94H	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455197	R01C01	COLO-94H
CX1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875081	R03C01	CX1
DiFi	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R05C02	DiFi
DLD1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R03C02	DLD1
FET	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R05C02	FET
GEO	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455251	R04C01	GEO
GP2d	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R05C02	GP2d
Gp5D	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R01C01	Gp5d
HCA24	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R02C01	HCA24
HCA46	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R02C01	HCA46
HCA7	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R04C02	HCA7
HCC2998	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R05C02	HCC2998
HCT116	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R04C02	HCT-116
HCT15	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R05C02	HCT-15
HCT8	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R06C01	HCT-8
HDC114	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455164	R05C01	HDC114
HDC135	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R03C01	HDC135
HDC142	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R02C01	HDC142
HDC143	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875089	R06C01	HDC143
HDC54	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455235	R03C02	HDC54
HDC8	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455199	R03C01	HDC8
HDC82	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R06C02	HDC82
HDC9	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455199	R02C01	HDC9
HRA16	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450147	R06C02	HRA16
HROC18	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R04C02	HROC18
HROC24	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R02C02	HROC24
HROC32	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R05C02	HROC32
HROC39	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R02C01	HROC39
HROC46	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455235	R01C01	HROC46
HROC69	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455251	R04C02	HROC69
HROC80	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455219	R06C01	HROC80
HROC87	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450134	R05C01	HROC87
HT115	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200486890054	R06C02	HT-115
HT29	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R01C01	HT-29
HT55	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R02C01	HT-55
HUTU80	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R02C01	HuTu80
KM12	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R03C01	KM-12
KM12C	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R03C01	KM-12C
KM12SM	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875089	R05C01	KM-12SM
KM20	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875081	R06C01	KM-20
LIM1215	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10011071041	R02C01	LIM-1215
LIM1899	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450134	R04C01	LIM-1899
LIM2099	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R06C01	LIM-2099
LIM2405	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455199	R02C02	LIM-2405
LIM2412	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450134	R02C01	LIM-2412
LIM2537	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455251	R05C02	LIM-2537
LIM2550	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R05C02	LIM-2550
LIM2551	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R01C01	LIM-2551
LoVo	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R03C02	LoVo
LS1034	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R06C02	LS1034
LS123	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R04C01	LS123
LS174T	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R06C02	LS174T
LS180	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R03C01	LS180
LS411N	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R03C01	LS411N
LS513	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R02C02	LS513
MDST8	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R04C01	MDST8
MIP101	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R01C02	MIP101
NCIH498	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875081	R04C01	NCI-H498
NCIH508	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R05C01	NCI-H508

NCIH630	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R05C01	NCI-H630
NCIH684	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R01C02	NCI-H684
NCIH716	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R05C01	NCI-H716
NCIH747	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R06C01	NCI-H747
OUMS23	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455199	R01C01	OUMS-23
OXCO1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455235	R01C02	OXCO1
OXCO2	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R03C02	OXCO2
OXCO3	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875118	R03C01	OXCO3
RCM1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R01C02	RCM1
RKO	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R01C01	RKO
RW7213	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455123	R04C01	RW7213
SKCO1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R02C02	SKCO-1
SNU1033	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455172	R01C02	SNU-1033
SNU1040	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R04C02	SNU-1040
SNU1047	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R02C01	SNU-1047
SNU1181	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875118	R04C01	SNU-1181
SNU1235	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450147	R04C01	SNU-1235
SNU1406	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R04C01	SNU-1406
SNU1411	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450147	R05C01	SNU-1411
SNU1460	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R06C02	SNU-1460
SNU1544	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R06C01	SNU-1544
SNU1684	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455225	R04C01	SNU-1684
SNU1746	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455225	R03C02	SNU-1746
SNU175	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R05C02	SNU-175
SNU254	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455254	R03C02	SNU-254
SNU283	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R04C01	SNU-283
SNU407	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R01C02	SNU-407
SNU479	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455123	R04C02	SNU-479
SNU503	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875089	R02C02	SNU-503
SNU61	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R02C01	SNU-61
SNU769B	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R03C01	SNU-769B
SNU81	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455164	R01C01	SNU-81
SNU977	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998998097	R03C02	SNU-977
SNUC1	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R05C01	SNUC-1
SNUC2A	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455164	R01C02	SNUC-2A
SNUC2B	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R02C02	SNUC-2B
SNUC4	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999876007	R04C01	SNUC-4
SNUC5	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R02C02	SNUC-5
SW1116	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R03C02	SW1116
SW1222	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647450134	R06C01	SW1222
SW1417	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R05C01	SW1417
SW1463	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R04C02	SW1463
SW403	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	10006823050	R03C01	SW403
SW48	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	6042324140	R02C01	SW48
SW480	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R05C02	SW480
SW620	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590019	R06C02	SW620
SW837	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426003	R04C02	SW-837
SW948	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R06C01	SW-948
T84	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	200693590020	R01C02	T84
V411	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455207	R03C02	V411
V457	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R03C02	V457
V481	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455251	R02C01	V481
V703	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999002152	R04C01	V703
VACO432	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R03C01	VACO-432
VACO5	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3999875081	R06C02	VACO-5
VACO6	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	9647455255	R01C01	VACO-6
WiDR	Cell_line	Colon_Adenocarcinoma_cell	GSE86078	3998426028	R04C01	WiDR

PUBLICALLY AVAILABLE DATA USED IN THE STUDY

Sample_Name	Tissue_type	Donor_type	Dataset	Sentrix_ID	Sentrix_Position
GSM796576	Normal_mucosa	Healthy	GSE32146		
GSM796577	Normal_mucosa	Healthy	GSE32146		
GSM796578	Normal_mucosa	Healthy	GSE32146		
GSM796579	Normal_mucosa	Healthy	GSE32146		
GSM796580	Normal_mucosa	Healthy	GSE32146		
GSM796581	Normal_mucosa	Healthy	GSE32146		
GSM796582	Normal_mucosa	Healthy	GSE32146		
GSM796583	Normal_mucosa	Healthy	GSE32146		
GSM796584	Normal_mucosa	Healthy	GSE32146		
GSM796585	Normal_mucosa	Healthy	GSE32146		
GSM796586	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796587	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796588	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796589	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796590	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796591	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796592	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796593	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796594	Normal_mucosa	Crohn's_disease	GSE32146		
GSM796598	Normal_mucosa	Crohn's_disease	GSE32146		
GSM1009660	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009666	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009667	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009668	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009673	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009674	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009677	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009681	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009685	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009686	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009687	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009688	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009689	Whole_Blood	Healthy_Blood	GSE41169		
GSM1009690	Whole_Blood	Healthy_Blood	GSE41169		

[illegible]

TCGA-AA-3494-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041065 R02C02
TCGA-AA-3495-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041088 R02C02
TCGA-AA-3502-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041065 R04C02
TCGA-AA-3506-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041007 R01C02
TCGA-AA-3509-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041084 R06C02
TCGA-AA-3510-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	5775041084 R03C02
TCGA-AA-3655-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042316015 R06C02
TCGA-AA-3660-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308165 R05C01
TCGA-AA-3663-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042316001 R05C02
TCGA-AA-3697-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308165 R04C02
TCGA-AA-3712-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042316011 R03C01
TCGA-AA-3713-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308165 R04C01
TCGA-AG-3725-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	6055424097 R02C02
TCGA-AG-3731-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	6055424075 R04C01
TCGA-AG-A01W-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	5775041086 R02C01
TCGA-AG-A01Y-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	5775041086 R05C01
TCGA-AG-A020-11	Normal_mucosa	Rectal_Mucinous_Adenocarcinoma_norm	TCGA COREAD	5775041086 R01C02
TCGA-AG-A02N-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	5775041086 R04C02
TCGA-AG-A036-11	Normal_mucosa	Rectal_Adenocarcinoma_norm	TCGA COREAD	5775041086 R06C02
TCGA-AZ-6598-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6057825035 R06C02
TCGA-AZ-6599-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6057825028 R03C02
TCGA-AZ-6600-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6057825020 R04C01
TCGA-AZ-6601-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6057825002 R01C02
TCGA-G4-6295-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308165 R06C02
TCGA-G4-6297-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042316015 R05C02
TCGA-G4-6298-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308159 R02C01
TCGA-G4-6302-11	Normal_mucosa	Colon_Mucinous_Adenocarcinoma_norm	TCGA COREAD	6042316001 R02C01
TCGA-G4-6311-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308159 R02C02
TCGA-G4-6314-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042316015 R01C02
TCGA-G4-6320-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6042308165 R02C01
TCGA-G4-6322-11	Normal_mucosa	Colon_Mucinous_Adenocarcinoma_norm	TCGA COREAD	6042308159 R01C01
TCGA-G4-6625-11	Normal_mucosa	Colon_Adenocarcinoma_norm	TCGA COREAD	6057825028 R04C02
TCGA-3L-AA1B-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R02C02
TCGA-4N-A93T-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R06C01
TCGA-4T-AA8H-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	3999492120 R06C01
TCGA-5M-AAT4-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492115 R06C02
TCGA-5M-AAT5-01	Tumor	NA	TCGA COREAD	3999492120 R05C01
TCGA-5M-AAT6-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R02C02
TCGA-5M-AATA-01	Tumor	NA	TCGA COREAD	3999492115 R05C02
TCGA-5M-AATE-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R06C02
TCGA-A6-2671-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R01C02
TCGA-A6-2675-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R04C01
TCGA-A6-2677-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216012 R01C02
TCGA-A6-2677-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216012 R02C02
TCGA-A6-2679-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R04C01
TCGA-A6-2680-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R01C01
TCGA-A6-2681-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R03C02
TCGA-A6-2682-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R06C01
TCGA-A6-2684-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R02C01
TCGA-A6-2684-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216012 R03C02
TCGA-A6-2684-01-3	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216012 R04C02
TCGA-A6-2685-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R06C01
TCGA-A6-2686-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R04C01
TCGA-A6-3809-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216012 R05C02
TCGA-A6-3809-01-2	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216012 R06C02
TCGA-A6-3810-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R01C01
TCGA-A6-3810-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R02C01
TCGA-A6-4105-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R03C02
TCGA-A6-4107-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R01C01
TCGA-A6-5656-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R06C01
TCGA-A6-5656-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R03C01
TCGA-A6-5656-01-3	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R04C01
TCGA-A6-5657-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R04C02
TCGA-A6-5659-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R01C01
TCGA-A6-5659-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R05C01
TCGA-A6-5659-01-3	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R06C01
TCGA-A6-5660-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R01C02
TCGA-A6-5661-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R06C01
TCGA-A6-5661-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	7796806109 R05C02
TCGA-A6-5662-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R01C01
TCGA-A6-5664-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R04C02
TCGA-A6-5665-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R02C02
TCGA-A6-5665-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	7796806109 R03C02
TCGA-A6-5666-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R01C02
TCGA-A6-5667-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R03C02
TCGA-A6-6137-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R01C01
TCGA-A6-6138-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R01C02
TCGA-A6-6140-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R03C02
TCGA-A6-6141-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R02C01
TCGA-A6-6142-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R01C02
TCGA-A6-6648-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R03C01
TCGA-A6-6649-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R06C02
TCGA-A6-6650-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R05C02
TCGA-A6-6650-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R01C02
TCGA-A6-6650-01-3	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9305216015 R02C02
TCGA-A6-6651-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R05C01
TCGA-A6-6652-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R06C01
TCGA-A6-6653-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R06C02
TCGA-A6-6654-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R02C01
TCGA-A6-6780-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718053 R04C01
TCGA-A6-6780-01-2	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216015 R03C02
TCGA-A6-6780-01-3	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216015 R04C02
TCGA-A6-6781-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509018 R01C01
TCGA-A6-6781-01-2	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509087 R05C02
TCGA-A6-6781-01-3	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216015 R05C02
TCGA-A6-6781-01-4	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9305216015 R06C02
TCGA-A6-6782-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R03C02

TCGA-A6-A565-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9296931074 R02C01
TCGA-A6-A566-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9296931073 R05C01
TCGA-A6-A567-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931074 R04C01
TCGA-A6-A56B-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R04C01
TCGA-A6-A5ZU-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R03C02
TCGA-AA-3488-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R04C01
TCGA-AA-3489-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R06C02
TCGA-AA-3492-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R06C01
TCGA-AA-3494-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R01C01
TCGA-AA-3495-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R01C01
TCGA-AA-3496-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R06C02
TCGA-AA-3502-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R06C02
TCGA-AA-3506-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R03C02
TCGA-AA-3509-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R04C02
TCGA-AA-3510-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R02C01
TCGA-AA-3511-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R05C01
TCGA-AA-3655-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R01C01
TCGA-AA-3660-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R06C02
TCGA-AA-3662-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R05C02
TCGA-AA-3663-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R04C02
TCGA-AA-3697-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R02C02
TCGA-AA-3712-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R02C02
TCGA-AA-3713-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R01C01
TCGA-AD-5900-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625011 R02C02
TCGA-AD-6548-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R05C01
TCGA-AD-6888-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R05C01
TCGA-AD-6888-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R03C02
TCGA-AD-6889-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R03C02
TCGA-AD-6889-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R04C02
TCGA-AD-6890-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R02C02
TCGA-AD-6890-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R01C01
TCGA-AD-6895-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R05C01
TCGA-AD-6899-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509087 R01C02
TCGA-AD-6901-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264496026 R06C02
TCGA-AD-6963-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R03C01
TCGA-AD-6963-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509127 R03C01
TCGA-AD-6964-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R02C01
TCGA-AD-6964-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R06C02
TCGA-AD-6965-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R05C02
TCGA-AD-6965-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R06C02
TCGA-AD-A5EJ-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R06C01
TCGA-AD-A5EK-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R02C02
TCGA-AF-2687-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R04C02
TCGA-AF-2690-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R01C02
TCGA-AF-2693-01	Tumor	NA	TCGA COREAD	6055424075 R04C02
TCGA-AF-3911-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R05C02
TCGA-AF-4110-01	Tumor	NA	TCGA COREAD	6055424097 R04C01
TCGA-AF-6136-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R03C01
TCGA-AF-6655-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R02C01
TCGA-AF-6672-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R06C02
TCGA-AF-A56K-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	9828653044 R03C02
TCGA-AF-A56L-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	9828653044 R04C02
TCGA-AF-A56N-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	9828653044 R05C02
TCGA-AG-3591-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R03C01
TCGA-AG-3592-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R03C02
TCGA-AG-3725-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R01C01
TCGA-AG-3731-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R04C02
TCGA-AG-3732-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R01C01
TCGA-AG-3742-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R02C02
TCGA-AG-4021-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R03C02
TCGA-AG-4022-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R06C02
TCGA-AG-A01W-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	5775041086 R01C01
TCGA-AG-A01Y-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	5775041086 R04C01
TCGA-AG-A020-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	5775041086 R06C01
TCGA-AG-A026-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	5775041086 R02C02
TCGA-AG-A02N-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	5775041086 R03C02
TCGA-AG-A036-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	5775041086 R05C02
TCGA-AH-6544-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R06C02
TCGA-AH-6547-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R05C02
TCGA-AH-6549-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R05C01
TCGA-AH-6643-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R06C02
TCGA-AH-6644-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R04C02
TCGA-AH-6897-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R05C01
TCGA-AH-6903-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	6929718114 R02C01
TCGA-AM-5820-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R03C02
TCGA-AM-5821-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R02C01
TCGA-AU-3779-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R04C01
TCGA-AU-6004-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R01C02
TCGA-AY-5543-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R06C02
TCGA-AY-6196-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042308165 R06C01
TCGA-AY-6197-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R06C02
TCGA-AY-6386-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R01C02
TCGA-AY-A54L-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R01C02
TCGA-AY-A69D-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R05C01
TCGA-AY-A71X-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R03C02
TCGA-AY-A8YK-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R03C01
TCGA-AZ-4308-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R03C01
TCGA-AZ-4313-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R05C02
TCGA-AZ-4315-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R02C02
TCGA-AZ-4323-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R06C01
TCGA-AZ-4614-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R01C02
TCGA-AZ-4615-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R03C01
TCGA-AZ-4616-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R03C01
TCGA-AZ-4681-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R01C02
TCGA-AZ-4682-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R05C01
TCGA-AZ-4684-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R05C02
TCGA-AZ-5403-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R02C01
TCGA-AZ-5407-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R04C02

TCGA-AZ-6598-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R04C02
TCGA-AZ-6599-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R05C01
TCGA-AZ-6600-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R02C02
TCGA-AZ-6601-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R05C01
TCGA-AZ-6603-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R01C02
TCGA-AZ-6605-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R01C02
TCGA-AZ-6606-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R01C02
TCGA-AZ-6607-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R03C02
TCGA-AZ-6608-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R05C02
TCGA-BM-6198-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R01C02
TCGA-CA-5254-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R01C01
TCGA-CA-5255-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R04C02
TCGA-CA-5256-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041084 R05C01
TCGA-CA-5796-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625037 R05C01
TCGA-CA-5797-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R06C01
TCGA-CA-6715-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R05C02
TCGA-CA-6716-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R02C01
TCGA-CA-6717-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718079 R05C01
TCGA-CA-6718-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R01C01
TCGA-CA-6719-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R01C02
TCGA-CI-6619-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R01C01
TCGA-CI-6620-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R03C02
TCGA-CI-6621-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R01C01
TCGA-CI-6622-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R06C01
TCGA-CI-6623-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R01C02
TCGA-CI-6624-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R04C02
TCGA-CK-4947-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R04C01
TCGA-CK-4948-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625068 R06C01
TCGA-CK-4950-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R03C01
TCGA-CK-4951-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	5775041084 R04C02
TCGA-CK-4952-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042308158 R03C01
TCGA-CK-5912-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R01C01
TCGA-CK-5913-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R05C01
TCGA-CK-5914-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R05C02
TCGA-CK-5915-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R01C02
TCGA-CK-5916-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R02C02
TCGA-CK-6746-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R02C02
TCGA-CK-6747-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R03C01
TCGA-CK-6748-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718079 R04C01
TCGA-CK-6751-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718054 R06C01
TCGA-CL-4957-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R03C02
TCGA-CL-5917-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R01C02
TCGA-CL-5918-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R05C01
TCGA-CM-4743-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R06C02
TCGA-CM-4744-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041065 R06C02
TCGA-CM-4746-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R05C01
TCGA-CM-4747-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R02C01
TCGA-CM-4748-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	5775041084 R03C01
TCGA-CM-4750-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041007 R06C01
TCGA-CM-4751-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R02C02
TCGA-CM-4752-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R03C01
TCGA-CM-5341-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	5775041088 R05C01
TCGA-CM-5344-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R06C01
TCGA-CM-5348-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R06C01
TCGA-CM-5349-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316011 R06C01
TCGA-CM-5860-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R05C02
TCGA-CM-5861-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R04C01
TCGA-CM-5862-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625068 R04C01
TCGA-CM-5863-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718053 R01C01
TCGA-CM-5864-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625068 R05C01
TCGA-CM-5868-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R04C01
TCGA-CM-6161-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R06C01
TCGA-CM-6162-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625068 R01C02
TCGA-CM-6163-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671148 R03C01
TCGA-CM-6164-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625068 R03C01
TCGA-CM-6165-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671148 R06C01
TCGA-CM-6166-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R05C01
TCGA-CM-6167-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671148 R05C01
TCGA-CM-6168-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671148 R04C01
TCGA-CM-6169-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R04C02
TCGA-CM-6170-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625057 R03C01
TCGA-CM-6171-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625037 R03C01
TCGA-CM-6172-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625068 R01C01
TCGA-CM-6674-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R03C02
TCGA-CM-6675-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R04C01
TCGA-CM-6676-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R02C01
TCGA-CM-6677-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R04C01
TCGA-CM-6678-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R04C02
TCGA-CM-6679-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R05C02
TCGA-CM-6680-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R06C02
TCGA-D5-5537-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R04C02
TCGA-D5-5537-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509127 R02C01
TCGA-D5-5538-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671148 R02C01
TCGA-D5-5539-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625011 R04C02
TCGA-D5-5540-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R03C01
TCGA-D5-5541-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625011 R02C01
TCGA-D5-6529-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R04C01
TCGA-D5-6530-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316011 R04C01
TCGA-D5-6531-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R05C02
TCGA-D5-6532-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R03C02
TCGA-D5-6533-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R02C01
TCGA-D5-6534-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509087 R02C01
TCGA-D5-6534-01-2	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509127 R05C01
TCGA-D5-6535-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316011 R01C01
TCGA-D5-6536-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R04C01
TCGA-D5-6537-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R03C02
TCGA-D5-6538-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R01C01
TCGA-D5-6539-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316011 R05C01

TCGA-D5-6540-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042316001 R01C02
TCGA-D5-6541-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R01C01
TCGA-D5-6898-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509087 R04C01
TCGA-D5-6898-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R06C01
TCGA-D5-6922-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509127 R01C01
TCGA-D5-6923-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509127 R04C01
TCGA-D5-6924-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R03C01
TCGA-D5-6924-01-2	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R06C02
TCGA-D5-6926-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509018 R01C02
TCGA-D5-6930-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509087 R02C02
TCGA-D5-7000-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6264509018 R04C01
TCGA-DC-4745-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R06C01
TCGA-DC-4749-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R06C02
TCGA-DC-5337-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R06C01
TCGA-DC-5869-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R06C01
TCGA-DC-6154-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718113 R03C02
TCGA-DC-6155-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R03C02
TCGA-DC-6156-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R02C02
TCGA-DC-6157-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R02C01
TCGA-DC-6158-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R04C02
TCGA-DC-6160-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R01C02
TCGA-DC-6681-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R03C02
TCGA-DC-6682-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R01C02
TCGA-DC-6683-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R05C02
TCGA-DM-A0X9-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316010 R04C02
TCGA-DM-A0XD-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316010 R06C02
TCGA-DM-A0XF-01	Tumor	NA	TCGA COREAD	6042316008 R02C01
TCGA-DM-A1D0-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R04C01
TCGA-DM-A1D4-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R06C01
TCGA-DM-A1D6-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042316008 R02C02
TCGA-DM-A1D7-01	Tumor	NA	TCGA COREAD	6042316008 R03C02
TCGA-DM-A1D8-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316010 R05C02
TCGA-DM-A1D9-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R01C01
TCGA-DM-A1DA-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R03C01
TCGA-DM-A1DB-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R05C01
TCGA-DM-A1HA-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316008 R01C02
TCGA-DM-A1HB-01	Tumor	NA	TCGA COREAD	6929718086 R01C02
TCGA-DM-A280-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625085 R04C02
TCGA-DM-A282-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929671163 R04C01
TCGA-DM-A285-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625085 R02C01
TCGA-DM-A288-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625085 R01C01
TCGA-DM-A28A-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R05C02
TCGA-DM-A28C-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R05C01
TCGA-DM-A28E-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R01C02
TCGA-DM-A28F-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R04C01
TCGA-DM-A28G-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R06C02
TCGA-DM-A28H-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625085 R06C01
TCGA-DM-A28K-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6285625085 R03C01
TCGA-DM-A28M-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6285625076 R06C02
TCGA-DT-5265-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	6929718081 R04C01
TCGA-DY-A0XA-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R02C01
TCGA-DY-A1DC-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R03C01
TCGA-DY-A1DD-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R04C01
TCGA-DY-A1DE-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R05C01
TCGA-DY-A1DF-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R06C01
TCGA-DY-A1DG-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R01C02
TCGA-DY-A1H8-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6264509055 R02C02
TCGA-EF-5830-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R05C02
TCGA-EF-5831-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718042 R06C02
TCGA-EI-6506-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R06C01
TCGA-EI-6507-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R03C01
TCGA-EI-6508-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R04C01
TCGA-EI-6509-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R02C02
TCGA-EI-6510-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R06C01
TCGA-EI-6511-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R02C02
TCGA-EI-6512-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R01C01
TCGA-EI-6513-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R05C01
TCGA-EI-6514-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424075 R02C01
TCGA-EI-6881-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R05C02
TCGA-EI-6882-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	6929718114 R03C02
TCGA-EI-6883-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R06C01
TCGA-EI-6884-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R02C02
TCGA-EI-6885-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R04C01
TCGA-EI-6917-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R01C02
TCGA-EI-7002-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718113 R05C02
TCGA-EI-7004-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	6929718113 R04C02
TCGA-F4-6459-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R03C01
TCGA-F4-6460-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825035 R05C02
TCGA-F4-6461-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R05C02
TCGA-F4-6463-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042308159 R03C01
TCGA-F4-6569-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R02C02
TCGA-F4-6570-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R06C01
TCGA-F4-6703-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718054 R02C02
TCGA-F4-6704-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6929718065 R03C02
TCGA-F4-6805-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R01C01
TCGA-F4-6806-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R02C01
TCGA-F4-6807-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718079 R03C01
TCGA-F4-6808-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R06C01
TCGA-F4-6809-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R02C02
TCGA-F4-6854-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6264509127 R06C01
TCGA-F5-6464-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R05C02
TCGA-F5-6465-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R05C01
TCGA-F5-6571-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R03C01
TCGA-F5-6702-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R04C01
TCGA-F5-6810-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718058 R05C02
TCGA-F5-6811-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R04C02
TCGA-F5-6812-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R02C02
TCGA-F5-6813-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718081 R06C01

TCGA-F5-6814-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718113 R06C02
TCGA-F5-6861-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R01C01
TCGA-F5-6863-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R03C01
TCGA-F5-6864-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718114 R04C02
TCGA-G4-6293-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R06C01
TCGA-G4-6294-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825035 R04C02
TCGA-G4-6295-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308165 R05C02
TCGA-G4-6297-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R05C01
TCGA-G4-6298-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R02C01
TCGA-G4-6299-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R04C01
TCGA-G4-6302-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042308158 R05C01
TCGA-G4-6303-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R01C01
TCGA-G4-6306-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R05C01
TCGA-G4-6307-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R04C01
TCGA-G4-6309-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718053 R05C02
TCGA-G4-6310-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R03C02
TCGA-G4-6311-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308159 R04C02
TCGA-G4-6314-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316011 R02C01
TCGA-G4-6315-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R06C01
TCGA-G4-6317-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R04C02
TCGA-G4-6320-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316015 R03C01
TCGA-G4-6321-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042308158 R01C02
TCGA-G4-6322-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	6042308158 R02C02
TCGA-G4-6323-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6042316001 R02C02
TCGA-G4-6586-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825020 R01C01
TCGA-G4-6588-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R06C01
TCGA-G4-6625-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825028 R02C01
TCGA-G4-6626-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R03C01
TCGA-G4-6627-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6057825002 R02C01
TCGA-G4-6628-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	6929718065 R04C02
TCGA-G5-6233-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424097 R02C01
TCGA-G5-6235-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6055424040 R05C01
TCGA-G5-6572-01	Tumor	Rectal_Adenocarcinoma	TCGA COREAD	6929718061 R03C02
TCGA-G5-6641-01	Tumor	Rectal_Mucinous_Adenocarcinoma	TCGA COREAD	6929718061 R02C01
TCGA-NH-A50T-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931074 R03C01
TCGA-NH-A50U-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9702496157 R05C02
TCGA-NH-A50V-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R02C01
TCGA-NH-A5IV-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R06C02
TCGA-NH-A6GA-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R01C02
TCGA-NH-A6GB-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R04C01
TCGA-NH-A6GC-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	3999492120 R04C01
TCGA-NH-A8F7-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R05C02
TCGA-NH-A8F8-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R01C02
TCGA-QG-A5YV-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931074 R05C01
TCGA-QG-A5YW-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	9296931073 R04C02
TCGA-QG-A5YX-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R03C01
TCGA-QG-A5Z1-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931074 R01C01
TCGA-QG-A5Z2-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9296931073 R05C02
TCGA-QL-A97D-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	3999492120 R02C01
TCGA-RU-A8FL-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R02C01
TCGA-SS-A7HO-01	Tumor	NA	TCGA COREAD	9702496157 R03C01
TCGA-T9-A92H-01	Tumor	Colon_Adenocarcinoma	TCGA COREAD	9702496157 R04C02
TCGA-WS-AB45-01	Tumor	Colon_Mucinous_Adenocarcinoma	TCGA COREAD	3999492120 R01C01
CCR141 CCR169	3331 Normal_mucosa	Healthy	GSE48684	
	3332 Normal_mucosa	Healthy	GSE48684	
	3333 Normal_mucosa	Healthy	GSE48684	
	3999 Normal_mucosa	Healthy	GSE48684	
	4000 Normal_mucosa	Healthy	GSE48684	
	4001 Normal_mucosa	Healthy	GSE48684	
	4002 Normal_mucosa	Healthy	GSE48684	
	4003 Normal_mucosa	Healthy	GSE48684	
	4004 Normal_mucosa	Healthy	GSE48684	
	4005 Normal_mucosa	Healthy	GSE48684	
	4006 Normal_mucosa	Healthy	GSE48684	
	3325 Normal_mucosa	Healthy	GSE48684	
	3326 Normal_mucosa	Healthy	GSE48684	
	3327 Normal_mucosa	Healthy	GSE48684	
	3328 Normal_mucosa	Healthy	GSE48684	
	3329 Normal_mucosa	Healthy	GSE48684	
	3330 Normal_mucosa	Healthy	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	5802 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	9043 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	9579 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	11827 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	11839 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	11879 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	3212 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	9047 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	11831 Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR221	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR209	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR281	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR289	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR253	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR197	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR193	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR217	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR185	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR181	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR233	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR139	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	
CCR277	Normal_mucosa	Colon_Adenocarcinoma_norm	GSE48684	

Supplementary File 2B

Differentially methylated probes identified with exclusion criteria

Probe_ID	CHR	MAPINFO	arm	gene	distancetoGene	feature	cgi	feat.cgi	conserved_tfbs	Exclusion criteria
cg09248054	1	969257	p	AGRN	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg27541454	1	975551	p	AGRN	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg22497741	1	1475644	p	C1orf70	NA	Body	shore	Body - shore	NA	Within telomere region
cg16306898	1	1475675	p	C1orf70	NA	1stExon	shore	1stExon - shore	NA	Within telomere region
cg16601494	1	1475737	p	C1orf70	NA	5'UTR	shore	5'UTR - shore	V\$P53_01	Positivity in blood
cg15487867	1	1475742	p	C1orf70	NA	TSS200	shore	TSS200 - shore	V\$P53_01	Within telomere region
cg24368383	1	1565856	p	MIB2	NA	Body	island	Body - island	V\$MYOGENF1_01	Not overlapping Differentially Methylated Region
cg04021697	1	3567303	p	WDR8	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg15472784	1	3663531	p	KIAA0495	NA	1stExon	island	1stExon - island	NA	Within telomere region
cg25467973	1	3663705	p	KIAA0495	NA	1stExon	island	1stExon - island	NA	Within telomere region
cg05121790	1	12123554	p	TNFRSF8	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg21819468	1	13910569	p	PDPN	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg17456100	1	22140859	p	LDLRAD2	NA	Body	shore	Body - shore	NA	Too far from other differentially methylated probes (>150bp)
cg15467646	1	22141014	p	LDLRAD2	NA	Body	island	Body - island	NA	Too far from other differentially methylated probes (>150bp)
cg24685006	1	36043014	p	TFAP2E	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg08599259	1	38510933	p	POU3F1	NA	3'UTR	island	3'UTR - island	NA	Not overlapping Differentially Methylated Region
cg23671221	1	44031300	p	PTPRF	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg04023150	1	44873064	p	RNF220	NA	5'UTR	island	5'UTR - island	NA	Too far from other differentially methylated probes (>150bp)
cg10224098	1	44873229	p	RNF220	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg16732616	1	50886782	p	DMRTA2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg19166660	1	57889035	p	DAB1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg05375728	1	58715539	p	DAB1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg27510182	1	58715553	p	DAB1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg02283366	1	63785544	p	FOXO3	-3186	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg25339566	1	67218165	p	TCTEX1D1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg14520424	1	70034491	p	PIN1P1	-191367	IGR	island	IGR - island	NA	Too far from other differentially methylated probes (>150bp)
cg08146483	1	75602412	p	LHX8	NA	Body	island	Body - island	NA	Positivity in blood
cg13463054	1	77333159	p	ST6GALNAC5	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg04077662	1	77333229	p	ST6GALNAC5	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg25026529	1	91183051	p	BARHL2	NA	TSS1500	shore	TSS1500 - shore	V\$USF_C	Not overlapping Differentially Methylated Region
cg24884703	1	91185422	p	BARHL2	7843	IGR	island	IGR - island	V\$E2F_02	Not overlapping Differentially Methylated Region
cg23233214	1	98511789	p	MIR137	NA	TSS200	shore	TSS200 - shore	NA	Not overlapping Differentially Methylated Region
cg14991984	1	99470129	p	LPPR5	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg06543087	1	108508548	p	VAV3	NA	TSS1500	shore	TSS1500 - shore	NA	Not overlapping Differentially Methylated Region
cg20302133	1	111217194	p	KCNA3	NA	1stExon	island	1stExon - island	NA	Within centromere region
cg26013553	1	111217406	p	KCNA3	NA	1stExon	island	1stExon - island	NA	Within centromere region
cg11595545	1	111217497	p	KCNA3	NA	1stExon	island	1stExon - island	NA	Within centromere region
cg01423964	1	111217575	p	KCNA3	NA	1stExon	island	1stExon - island	V\$NRSF_01	Within centromere region
cg06750832	1	111217691	p	KCNA3	NA	TSS200	island	TSS200 - island	NA	Within centromere region
cg07808555	1	111217712	p	KCNA3	NA	TSS200	island	TSS200 - island	NA	Within centromere region
cg14175690	1	119527638	p	TBX15	NA	5'UTR	shore	5'UTR - shore	NA	Positivity in blood
cg01439876	1	119543336	p	WARS2	-30503	IGR	island	IGR - island	NA	Positivity in blood
cg01959730	1	119548825	p	WARS2	-25014	IGR	shore	IGR - shore	NA	Positivity in blood
cg02457680	1	154475139	p	TDRD10	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg23089825	1	170630558	p	PRRX1	-2755	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg16993043	1	200008026	p	NRS5A2	NA	Body	shore	Body - shore	NA	Not overlapping Differentially Methylated Region
cg09557387	1	207818395	p	CRYL	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg07914084	1	237205999	p	RYR2	NA	Body	island	Body - island	NA	Positivity in blood
cg02328010	1	237206482	p	RYR2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg18663333	2	5833195	p	SOX11	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg04043795	2	5836366	p	SOX11	NA	3'UTR	island	3'UTR - island	NA	Not overlapping Differentially Methylated Region
cg06333058	2	29338077	p	CLIP4	NA	TSS1500	island	TSS1500 - island	NA	Selected probes
cg25737323	2	29338100	p	CLIP4	NA	TSS1500	island	TSS1500 - island	V\$SPZ1_01	Selected probes
cg23428985	2	29338113	p	CLIP4	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg23255835	2	29338121	p	CLIP4	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg03135351	2	29338258	p	CLIP4	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg08808128	2	29338432	p	CLIP4	NA	1stExon	island	1stExon - island	NA	Too far from other differentially methylated probes (>150bp)
cg16439198	2	38203674	p	CYP11B1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg14411266	2	40679582	p	SLC8A1	NA	5'UTR	shore	5'UTR - shore	NA	Not overlapping Differentially Methylated Region
cg22884656	2	45157296	p	SIX3	-11741	IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg23325963	2	45160093	p	SIX3	-8944	IGR	island	IGR - island	NA	Positivity in blood
cg03714619	2	45160445	p	SIX3	-8592	IGR	shore	IGR - shore	NA	Positivity in blood
cg02155398	2	45160490	p	SIX3	-8547	IGR	shore	IGR - shore	NA	Positivity in blood
cg00097146	2	45171818	p	SIX3	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg17526573	2	50574708	p	NRXN1	NA	Body	island	Body - island	NA	Positivity in blood
cg27364741	2	63281069	p	OTX1	NA	Body	island	Body - island	NA	Selected probes
cg25622366	2	63281139	p	OTX1	NA	Body	island	Body - island	NA	Selected probes
cg04654530	2	63282702	p	OTX1	NA	Body	island	Body - island	NA	Positivity in blood
cg07974511	2	63283013	p	OTX1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg21472506	2	63283967	p	OTX1	NA	3'UTR	island	3'UTR - island	NA	Selected probes
cg23229261	2	63284066	p	OTX1	NA	3'UTR	island	3'UTR - island	NA	Selected probes
cg10122865	2	63284132	p	OTX1	NA	3'UTR	island	3'UTR - island	NA	Selected probes
cg11536474	2	63286049	p	OTX1	8112	IGR	island	IGR - island	NA	Positivity in blood
cg11573679	2	68546467	p	CNRIP1	NA	1stExon	island	1stExon - island	NA	Selected probes
cg07080358	2	68546507	p	CNRIP1	NA	1stExon	island	1stExon - island	NA	Selected probes
cg24171907	2	68546579	p	CNRIP1	NA	1stExon	island	1stExon - island	NA	Selected probes
cg19656282	2	74742786	p	TLX2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg21185289	2	74743437	p	TLX2	NA	3'UTR	island	3'UTR - island	NA	Not overlapping Differentially Methylated Region
cg08189989	2	105459164	p	POU3F3	-12805	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17529832	2	105461096	p	POU3F3	-10873	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg21858380	2	119916486	p	C1QL2	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg07212778	2	119916510	p	C1QL2	NA	TSS200	island	TSS200 - island	NA	Too far from other differentially methylated probes (>150bp)
cg15975865	2	127413831	p	GYPC	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg19484402	2	127414108	p	GYPC	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg26820055	2	131721099	p	ARHGFE4	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg26210445	2	162273326	p	TBR1	NA	1stExon	island	1stExon - island	NA	Positivity in blood
cg20935165	2	172972840	p	DLX2	8674	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg06952671	2	182322268	p	ITGA4	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg21995919	2	182322279	p	ITGA4	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg25024074	2	182322501	p	ITGA4	NA	1stExon	island	1stExon - island	NA	Too far from other differentially methylated probes (>150bp)
cg11947981	2	182322749	p	ITGA4	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg16918905	2	220361609	p	GMPPA	-1978	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg00793935	2	234847769	p	TRPM8	NA	Body	island	Body - island	NA	Positivity in blood
cg10429608	2	238864632	p	UBE2F-SCLY	-10955	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg23141355	3	44063593	p	MIR138-1	-92111	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg22702772	3	48699012	p	CELSR3	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg20072171	3	62356962	p	FEZF2	NA	Body	island	Body - island	V\$FAC1_01	Not overlapping Differentially Methylated Region
cg08668199	3	127795571	p	RUVBL1	-4229	IGR	island	IGR - island	NA	Positivity in blood
cg11940285	3	129693385	p	TRH	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg22512438	3	129693489	p	TRH	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg02700891	3	129693586	p	TRH	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg17373442	3	142839991	p	CHST2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg12892506	3	147113918	p	ZIC4	NA	Body	island	Body - island	NA	Positivity in blood
cg14768785	3	172166517	p	GHSR	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg07393736	3	172167810	p	GHSR	2477	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg02119363	3	179754603	p	PEX5L	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg13473356	3	179754613	p	PEX5L	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg18780412	3	179755086	p	PEX5L	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg24000814	3	184301730	p	EPHB3	22143	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg16376000	3	192127330	p	FGF12	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg17393267	3	192127356	p	FGF12	NA	TSS1500	island	TSS1500 - island	NA	Within telomere region
cg08002883	3	192127457	p	FGF12	NA	TSS1500	island	TSS1500 - island	NA	Within telomere region
cg22834653	3	192232077	p	FGF12	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg17128947	4	779480	p	CPLX1	NA	3'UTR	island	3'UTR - island	NA	Positivity in blood
cg17397631	4	779880	p	CPLX1	NA	3'UTR	island	3'UTR - island	NA	Positivity in blood
cg27655158	4	1396593	p	CRIPAK	11253	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg14602530	4	4859772	p	MSX1	-1620	IGR	island	IGR - island	V\$IK1_01;V\$IK3_01	Positivity in blood
cg15092343	4	4860061	p	MSX1	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood

cg23300732	4	5053496	p	STK32B	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg08323075	4	5053504	p	STK32B	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg04575395	4	5892072	p	CRMP1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg18790597	4	17783331	p	FAM184B	NA	TSS200	island	TSS200 - island	NA	Too far from other differentially methylated probes (>150bp)
cg07666699	4	17783502	p	FAM184B	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg07907386	4	41749443	p	PHOX2B	NA	Body	island	Body - island	NA	Positivity in blood
cg24657817	4	42153708	p	BEND4	NA	Body	island	Body - island	V\$MYOGNF1_01	Not overlapping Differentially Methylated Region
cg23209990	4	55097576	q	PDGFRA	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg05754435	4	55992155	q	KDR	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg13139972	4	107957430	q	DKK2	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg04188273	4	110223830	q	COL25A1	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg21077559	4	122686319	q	TMEM155	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg04638468	4	122686453	q	TMEM155	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg08553437	4	122686456	q	TMEM155	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg07978472	4	122686493	q	TMEM155	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg23901852	4	126238384	q	FAT4	NA	1stExon	island	1stExon - island	V\$TAXCREB_01	Not overlapping Differentially Methylated Region
cg10196720	4	134069593	q	PCDH10	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg17504999	4	134072723	q	PCDH10	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg03112087	4	142053720	q	RNF150	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg15042811	4	144621971	q	FREM3	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg24199834	4	147560126	q	POU4F2	NA	1stExon	island	1stExon - island	NA	Positivity in blood
cg16887264	4	147561775	q	POU4F2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg00699993	4	158141570	q	GRIA2	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg26814276	4	172734266	q	GALNTL6	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg15707093	4	174450353	q	NBLA00301	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg14732324	5	528621	p	MIR4456		-7334	IGR	IGR - island	NA	Within telomer region
cg14564616	5	1876337	p	IRX4		-1204	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg06585708	5	3602413	p	IRX1		6245	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg08466792	5	3603227	p	IRX1		7059	IGR	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg25092681	5	16180033	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg00339556	5	16180048	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg01791874	5	16180055	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg17030173	5	16180062	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg17712694	5	16180068	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg16150752	5	16180072	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg21901718	5	16180076	p	MARCH11	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg23065934	5	16180266	p	MARCH11	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg10932018	5	54516487	q	MCIDAS		1062	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18456523	5	54516805	q	MCIDAS		1380	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg14608384	5	54516879	q	MCIDAS		1454	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18250028	5	71015523	q	CARTPT	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg17448335	5	76249776	q	CRHBP	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg04502985	5	77268452	q	AP3B1		-29698	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg23180938	5	115152485	q	CDO1	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg08516516	5	115152492	q	CDO1	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg11036833	5	115152494	q	CDO1	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg22488797	5	134363324	q	PITX1		-100	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18443359	5	134374693	q	PITX1		11269	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg01283246	5	135266135	q	FBXL21	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg24847829	5	136834464	q	SPOCK1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg14650610	5	136834492	q	SPOCK1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg23445461	5	140864733	q	PCDHGA4	NA	Body	island	Body - island	NA	Positivity in blood
cg20755170	5	145720024	q	POU4F3	NA	3'UTR	island	3'UTR - island	NA	Not overlapping Differentially Methylated Region
cg07136988	5	168728081	q	SLIT3	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg18705773	5	170743564	q	TLX3		7276	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg05560435	5	172671526	q	NKX2-5		12419	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17228900	6	391764	p	IRF4	NA	5'UTR	island	5'UTR - island	NA	Within telomer region
cg06392169	6	391936	p	IRF4	NA	5'UTR	island	5'UTR - island	NA	Within telomer region
cg21277995	6	393239	p	IRF4	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg00110654	6	10385489	p	TFAP2A-AS1		-11427	IGR	IGR - island	NA	Positivity in blood
cg24452128	6	10390919	p	TFAP2A-AS1		-5997	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17754510	6	10391412	p	TFAP2A-AS1		-5504	IGR	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg05452406	6	10881891	p	GCM2	NA	Body	island	Body - island	NA	Positivity in blood
cg10903903	6	27647843	p	LINC01012		-13971	IGR	IGR - island	NA	Positivity in blood
cg15490715	6	29521568	p	UBD		-1821	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg22617773	6	29521751	p	UBD		-1638	IGR	IGR - island	NA	Selected probes
cg14278853	6	29521756	p	UBD		-1633	IGR	IGR - island	NA	Selected probes
cg17394649	6	29760164	p	HCG4	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg27011480	6	30228083	p	HLA-L	NA	Body	island	Body - island	NA	Positivity in blood
cg27200446	6	41606439	p	MDF1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg01883425	6	41606770	p	MDF1	NA	Body	shore	Body - shore	NA	Not overlapping Differentially Methylated Region
cg18884037	6	62996214	q	KHDRBS2	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg05447008	6	73331114	q	KCNQ5	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg00852573	6	73331405	q	KCNQ5	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg16334314	6	84418789	p	SNAPR1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg14367020	6	99292286	q	POU3F2		9706	IGR	IGR - shore	NA	Positivity in blood
cg17386213	6	108488335	p	NR2E1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg05085230	6	133562461	q	EY44	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg08712932	6	133562463	q	EY44	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg11664500	6	133562479	q	EY44	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg14287112	6	133562485	q	EY44	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg22871668	6	133562492	q	EY44	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg00356183	7	751833	p	PRKAR1B	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg08558397	7	752149	p	PRKAR1B	NA	5'UTR	island	5'UTR - island	NA	Within telomer region
cg16305865	7	752180	p	PRKAR1B	NA	5'UTR	shore	5'UTR - shore	NA	Within telomer region
cg20381963	7	752238	p	PRKAR1B	NA	5'UTR	shore	5'UTR - shore	NA	Positivity in blood
cg18601167	7	752286	p	PRKAR1B	NA	5'UTR	shore	5'UTR - shore	NA	Within telomer region
cg13895235	7	752292	p	PRKAR1B	NA	5'UTR	shore	5'UTR - shore	NA	Within telomer region
cg23244488	7	19146032	p	TWIST1		-9059	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg02400740	7	19157938	p	TWIST1	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg24885417	7	24323764	p	NPY	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg16964348	7	24323799	p	NPY	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg25884711	7	24323840	p	NPY	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg21097881	7	24323939	p	NPY	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg24245418	7	24324976	p	NPY	NA	Body	island	Body - island	NA	Positivity in blood
cg21773872	7	30722320	p	CRHR2	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg05809668	7	35301188	p	TBX20		30076	IGR	IGR - island	NA	Positivity in blood
cg17093995	7	49815502	p	VWC2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg19868631	7	54609776	p	VSTM2A	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg02300154	7	70597058	q	WBSCR17	NA	TSS1500	island	TSS1500 - island	NA	Too far from other differentially methylated probes (>150bp)
cg03044249	7	70597065	q	WBSCR17	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg05223720	7	71801793	q	CALN1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg12973591	7	93519473	q	TFPI2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg20230721	7	93519855	q	TFPI2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg16934178	7	93520074	q	TFPI2	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg01307939	7	98467571	q	TMEM130	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg07697895	7	116963259	q	WNT2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg04153784	7	127672235	q	SDN1	NA	Body	island	Body - island	NA	Too far from other differentially methylated probes (>150bp)
cg12628196	7	127672458	q	SDN1	NA	Body	island	Body - island	NA	Positivity in blood
cg09087503	7	127672473	q	SDN1	NA	Body	island	Body - island	NA	Selected probes
cg09296001	7	127672564	q	SDN1	NA	Body	island	Body - island	NA	Selected probes
cg12345672	7	127672658	q	SDN1	NA	Body	island	Body - island	NA	Selected probes
cg03514404	7	143579665	q	FAM115A	NA	5'UTR	shelf	5'UTR - shelf	NA	Selected probes
cg01303504	7	143579698	q	FAM115A	NA	5'UTR	shelf	5'UTR - shelf	NA	Selected probes
cg03225210	7	143579951	q	FAM115A	NA	5'UTR	shelf	5'UTR - shelf	NA	Too far from other differentially methylated probes (>150bp)
cg02864844	7	149917263	q	ACTR3C		-27038	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region
cg21532325	7	151107400	q	WDR86	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg19814400	7	158936632	q	VIPR2	NA	Body	island	Body - island	NA	Within telomer region
cg21038156	7	158936739	q	VIPR2	NA	Body	island	Body - island	NA	Within telomer region
cg18349835	7	158937107	q	VIPR2	NA	Body	island	Body - island	NA	Within telomer region
cg03976877	7	158937610	q	VIPR2	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg01200640	8	687384	p	ERICH1		73184	IGR	IGR - island	NA	Not overlapping Differentially Methylated Region

cg26777883	8	688417	p	ERICH1		74217	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg04218812	8	9763263	p	MIR124-1		2365	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18577280	8	16884549	p	EFHA2	NA			island	TSS200 - island	NA	Too far from other differentially methylated probes (>150bp)
cg19677607	8	24772270	p	NEFM	NA			island	TSS200 - island	V\$LMO2COM_01	Not overlapping Differentially Methylated Region
cg04118306	8	24772350	p	NEFM	NA			island	TSS200 - island	NA	Selected probes
cg03169018	8	24772435	p	NEFM	NA			island	TSS200 - island	NA	Selected probes
cg04555373	8	31497042	p	NRG1	NA			island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg24946597	8	31497464	p	NRG1	NA			5UTR	5UTR - island	NA	Positivity in blood
cg21517947	8	41167107	p	SFRP1	NA			island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg05166490	8	41754172	p	ANK1	NA			1stExon	1stExon - island	NA	Within centromer region
cg17331296	8	41754181	p	ANK1	NA			1stExon	1stExon - island	NA	Within centromer region
cg15531403	8	53852184	q	NPBWR1	NA			island	TSS1500 - island	NA	Positivity in blood
cg07770968	8	53852422	q	NPBWR1	NA			island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg16491617	8	54164391	q	OPRK1	NA			island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg11071231	8	57069907	q	PLAG1		-3561	IGR	island	IGR - island	NA	Selected probes
cg16504626	8	57070013	q	PLAG1		-3455	IGR	island	IGR - island	NA	Selected probes
cg04612444	8	57358713	q	PENK	NA			5UTR	5UTR - island	NA	Positivity in blood
cg25260137	8	65282185	q	LINC00966		-3590	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg25900085	8	65291513	q	MIR124-2	NA			shore	TSS200 - shore	NA	Positivity in blood
cg18065361	8	67344588	q	ADHFE1	NA			island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg20295442	8	67344665	q	ADHFE1	NA			island	TSS200 - island	NA	Selected probes
cg20912169	8	67344720	q	ADHFE1	NA			5UTR	5UTR - island	NA	Selected probes
cg14353137	8	67873799	q	TCF24		15063	IGR	island	IGR - island	NA	Too far from other differentially methylated probes (>150bp)
cg20980783	8	67874178	q	TCF24		15442	IGR	island	IGR - island	NA	Selected probes
cg26618965	8	67874206	q	TCF24		15470	IGR	island	IGR - island	NA	Selected probes
cg22001496	8	69243486	q	MIR548H4		529	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg21479226	8	69244510	q	MIR548H4		1553	IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg25832771	8	72756058	q	MSC	NA			1stExon	1stExon - island	NA	Selected probes
cg09734791	8	72756155	q	MSC	NA			1stExon	1stExon - island	V\$E47_01	Selected probes
cg05690644	8	97158015	q	GDF6	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg13211683	8	97171827	q	GDF6	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg00421139	8	97172961	q	GDF6	NA			1stExon	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg18233405	8	98290148	q	TSPLY5	NA			1stExon	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg16462462	8	117950825	q	C8orf85	NA			Body	Body - shore	NA	Positivity in blood
cg13912117	8	132054555	q	ADCY8		262008	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17698295	8	145106299	q	OPLAH	NA			Body	Body - island	NA	Within telomer region
cg17301223	8	145106438	q	OPLAH	NA			Body	Body - island	NA	Within telomer region
cg22882523	8	145107012	q	OPLAH	NA			Body	Body - island	NA	Positivity in blood
cg14058647	9	88137909	q	AGTPBP1		-23545	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg24393316	9	100616469	q	FOXE1	NA			1stExon	1stExon - island	V\$TAXCREB_01	Positivity in blood
cg24039697	10	8094534	p	FLJ45983				Body	Body - island	NA	Positivity in blood
cg18794404	10	22542024	p	LOC100130992		1023	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg05099508	10	22634432	p	SPAG6	NA			1stExon	1stExon - island	NA	Selected probes
cg24031355	10	22634439	p	SPAG6	NA			1stExon	1stExon - island	NA	Selected probes
cg23068913	10	23463377	p	PTF1A		-18083	IGR	island	IGR - island	NA	Positivity in blood
cg24487076	10	23983496	p	KIAA1217	NA			TSS200	TSS200 - island	NA	Selected probes
cg13882278	10	23983498	p	KIAA1217	NA			TSS200	TSS200 - island	NA	Selected probes
cg08539841	10	23983538	p	KIAA1217	NA			TSS200	TSS200 - island	V\$AHRARNT_01	Not overlapping Differentially Methylated Region
cg07825347	10	43600497	q	RET	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg06365057	10	50323706	q	C10orf72	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg20744625	10	64578469	q	EGR2	NA			5UTR	5UTR - island	NA	Not overlapping Differentially Methylated Region
cg06419761	10	72043661	q	LRRC20		-15068	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg11036831	10	82116382	q	DYDC2	NA			TSS200	TSS200 - island	NA	Too far from other differentially methylated probes (>150bp)
cg03701427	10	82117089	q	DYDC1	NA			TSS1500	TSS1500 - island	NA	Positivity in blood
cg05316043	10	83634974	q	NRG3	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg17538572	10	94834763	q	CYP26A1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg17863743	10	100993587	q	HPSE2	NA			Body	Body - shore	NA	Not overlapping Differentially Methylated Region
cg12118269	10	102893980	q	TLX1	NA			Body	Body - island	NA	Selected probes
cg24812837	10	102894120	q	TLX1	NA			Body	Body - island	NA	Selected probes
cg14861089	10	102895043	q	TLX1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg05482942	10	102899285	q	TLX1		8224	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg14038391	10	102900130	q	TLX1		9069	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17101450	10	102900365	q	TLX1		9304	IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg25209842	10	103536342	q	FGF8	NA			TSS1500	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg23095743	10	104000831	p	PITX3	NA			5UTR	5UTR - island	NA	Positivity in blood
cg21384402	10	105036701	q	INA	NA			TSS1500	TSS1500 - island	NA	Selected probes
cg24680586	10	105036727	q	INA	NA			TSS200	TSS200 - island	NA	Selected probes
cg18932798	10	105037503	q	INA	NA			1stExon	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg02320740	10	106400259	q	SORCS3	NA			TSS1500	TSS1500 - island	NA	Too far from other differentially methylated probes (>150bp)
cg14752336	10	106400454	q	SORCS3	NA			TSS1500	TSS1500 - island	NA	Too far from other differentially methylated probes (>150bp)
cg03958798	10	106400686	q	SORCS3	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg13302291	10	118030970	q	GFRA1	NA			Body	Body - island	V\$OLF1_01;V\$ROAZ_01	Not overlapping Differentially Methylated Region
cg05667348	10	118892581	q	VAX1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg24390913	10	118899788	q	VAX1		6987	IGR	island	IGR - island	NA	Positivity in blood
cg19912142	10	119494671	q	EMX2OS		192715	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg14072515	10	125732604	q	CHST15		-34578	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg08701047	10	130085199	q	MKI67		190274	IGR	open sea	IGR - open sea	NA	Not overlapping Differentially Methylated Region
cg27513573	10	131768098	q	CTAGE7P		-94064	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg03109827	10	133110349	q	TCERG1L	NA			TSS1500	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg02886408	10	133796164	q	BNIP3	NA			TSS1500	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg12816961	10	134559827	q	NKX6-2	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg25774643	11	627175	p	SCT	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg12928379	11	637175	p	DRD4	NA			TSS200	TSS200 - island	NA	Within telomer region
cg09607276	11	637491	p	DRD4	NA			1stExon	1stExon - island	NA	Within telomer region
cg25098208	11	8190659	p	RIC3	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg02027945	11	20618230	p	SLC6A5		-2716	IGR	island	IGR - island	NA	Positivity in blood
cg01642521	11	20618250	p	SLC6A5		-2696	IGR	island	IGR - island	NA	Positivity in blood
cg14749465	11	20690720	p	NELL1	NA			TSS1500	TSS1500 - island	NA	Selected probes
cg02510267	11	20690807	p	NELL1	NA			TSS1500	TSS1500 - island	NA	Selected probes
cg23861668	11	20691126	p	NELL1	NA			5UTR	5UTR - island	NA	Selected probes
cg14689623	11	20691161	p	NELL1	NA			5UTR	5UTR - island	NA	Selected probes
cg01563031	11	20691429	p	NELL1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg09537620	11	31826574	p	PAX6	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg18372607	11	31827084	p	PAX6	NA			Body	Body - shore	NA	Not overlapping Differentially Methylated Region
cg26848718	11	32454975	p	WT1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg15556502	11	43602845	p	MIR129-2				TSS200	TSS200 - island	NA	Selected probes
cg14416371	11	43602847	p	MIR129-2	NA			TSS200	TSS200 - island	NA	Selected probes
cg14944647	11	43602857	p	MIR129-2	NA			TSS200	TSS200 - island	NA	Selected probes
cg01939477	11	43602879	p	MIR129-2	NA			TSS200	TSS200 - island	NA	Selected probes
cg05376374	11	43602920	p	MIR129-2	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg09802835	11	71952131	q	PHOX2A	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg04543008	11	71955332	q	PHOX2A	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg03225817	11	105481317	q	GRIA4	NA			5UTR	5UTR - island	NA	Selected probes
cg04747226	11	105481319	q	GRIA4	NA			5UTR	5UTR - island	NA	Selected probes
cg07972135	11	105481322	q	GRIA4	NA			5UTR	5UTR - island	NA	Selected probes
cg22879515	11	111383515	q	MIR34B	NA			TSS200	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg09657963	11	124790875	q	HEPACAM	NA			3UTR	3UTR - island	NA	Positivity in blood
cg03419885	11	125036385	q	PKNOX2	NA			5UTR	5UTR - island	NA	Not overlapping Differentially Methylated Region
cg23727983	11	125774082	q	DDX25	NA			TSS200	TSS200 - shore	NA	Selected probes
cg01736784	11	125774092	q	DDX25	NA			TSS200	TSS200 - shore	NA	Selected probes
cg11017065	11	128564874	q	FLI1	NA			Body	Body - island	NA	Not overlapping Differentially Methylated Region
cg07072722	11	131781246	q	NTM	NA			1stExon	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg06947913	12	50297793	q	FAIM2	NA			TSS200	TSS200 - island	NA	Too far from other differentially methylated probes (>150bp)
cg00817367	12	52401214	q	GRASP	NA			Body	Body - island	NA	Too far from other differentially methylated probes (>150bp)
cg09597070	12	54088972	q	CALCOCO1		-15930	IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg08190858	12	54321346	q	HOXC13-AS		-7766	IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg27277463	12	62585031	q	FAM19A2	NA			5UTR	5UTR - island	NA	Not overlapping Differentially Methylated Region
cg26272220	12	81471884	q	ACSS3	NA			1stExon	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg22538054	12	95941988	q	USP44	NA			5UTR	5UTR - island	NA	Not overlapping Differentially Methylated Region
cg03308628	12										

cg03817911	12	104697389	q	TXNRD1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg10790429	12	106974623	q	LOC100287944		-2062 IGR	island	IGR - island	NA	Positivity in blood
cg11213520	12	113901529	q	LHX5	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg04569082	12	127940323	q	FLJ37505		-425839 IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg09170112	12	132102188	q	SFSWAP		-93444 IGR	open sea	IGR - open sea	NA	Positivity in blood
cg04865180	13	23734385	q	SGCG		-20675 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg19937061	13	25320133	q	RNF17		-18168 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg25366582	13	25621027	q	PABPC3		-49249 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg20194314	13	28503060	q	PDX1		8892 IGR	island	IGR - island	NA	Positivity in blood
cg17200768	13	28503373	q	PDX1		9205 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg02478448	13	37006063	q	CCNA1	NA	1stExon	island	1stExon - island	NA	Positivity in blood
cg18348647	13	37006107	q	CCNA1	NA	1stExon	island	1stExon - island	NA	Too far from other differentially methylated probes (>150bp)
cg08866608	13	58203798	q	PCDH17		-1991 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg20634967	13	58204224	q	PCDH17		-1565 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg23766591	13	78493205	q	EDNRB	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg24236409	13	78493282	q	EDNRB	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg26132774	13	79170146	q	RNF219-AS1		-3084 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg00295794	13	100641409	q	ZIC2		7383 IGR	island	IGR - island	NA	Positivity in blood
cg18115507	13	102069169	q	NALCN	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg11437784	13	112710823	q	SOX1		-11090 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg13692446	13	112759719	q	SOX1		37806 IGR	island	IGR - island	NA	Within telomere region
cg25570913	13	112759893	q	SOX1		37980 IGR	island	IGR - island	NA	Within telomere region
cg15384598	14	24045549	q	JPH4	NA	Body	island	Body - island	V\$ER_Q6	Not overlapping Differentially Methylated Region
cg08217024	14	48145108	q	MDGA2	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg19871940	14	57264287	q	OTX2		-3138 IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg21026830	14	60973517	q	SIX6		-2421 IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg06785999	14	60975964	q	SIX6	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg19456540	14	60976285	q	SIX6	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg04281464	14	70014873	q	CCDC177		-21658 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg23241781	14	70653964	q	SLC8A3	NA	5'UTR	shore	5'UTR - shore	NA	Not overlapping Differentially Methylated Region
cg22916722	14	76605056	q	GPATCH2L		-13203 IGR	island	IGR - island	NA	Too far from other differentially methylated probes (>150bp)
cg17410236	14	85996495	q	FLRT2	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg02874376	14	101193397	q	DLK1		1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg03419058	15	26108391	q	ATP10A		TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg10652393	15	27112430	q	GABRA5	NA	1stExon	island	1stExon - island	V\$TAXCREB_01	Not overlapping Differentially Methylated Region
cg04803843	15	28351906	q	HERC2		-4277 IGR	island	IGR - island	NA	Too far from other differentially methylated probes (>150bp)
cg03061682	15	28352098	q	HERC2		-4085 IGR	island	IGR - island	NA	Too far from other differentially methylated probes (>150bp)
cg00330492	15	28352558	q	HERC2		-3625 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg17741501	15	29077493	q	MIR5009		-12614 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg13800209	15	37390284	q	MEIS2	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg02018277	15	65116255	q	PIF1	NA	Body	island	Body - island	NA	Positivity in blood
cg09193347	15	79381867	q	RASGRF1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg20973720	15	79383167	q	RASGRF1	NA	5'UTR	island	5'UTR - island	V\$TAXCREB_02	Not overlapping Differentially Methylated Region
cg11167100	15	83776269	q	TM6SF1	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg08452658	15	83776271	q	TM6SF1	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg26460092	15	83776420	q	TM6SF1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg03063639	15	83776422	q	TM6SF1	NA	5'UTR	island	5'UTR - island	NA	Positivity in blood
cg02318926	15	83952345	q	BNC1	NA	Body	island	Body - island	NA	Selected probes
cg23989963	15	83952420	q	BNC1	NA	Body	island	Body - island	NA	Selected probes
cg17124224	15	83953880	q	BNC1	NA	TSS1500	island	TSS1500 - island	NA	Too far from other differentially methylated probes (>150bp)
cg06523224	15	83953883	q	BNC1	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg19288904	15	98836248	q	FAM169B		-144143 IGR	open sea	IGR - open sea	NA	Positivity in blood
cg05915293	16	2041512	p	SYNGR3	NA	shore	Body	Body - shore	NA	Not overlapping Differentially Methylated Region
cg07642043	16	10276674	p	GRIN2A	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg00133595	16	13228059	p	TRIM72	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg02809746	16	49312033	q	CBLN1		204 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg04698114	16	51184379	q	SALL1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg08806408	16	51185001	q	SALL1	NA	TSS1500	island	TSS1500 - island	NA	Selected probes
cg00124695	16	51185039	q	SALL1	NA	TSS1500	island	TSS1500 - island	NA	Selected probes
cg08439930	16	51185060	q	SALL1	NA	TSS1500	island	TSS1500 - island	NA	Positivity in blood
cg06653699	16	51185082	q	SALL1	NA	TSS1500	island	TSS1500 - island	NA	Selected probes
cg07502439	16	51185461	q	SALL1	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg01064265	16	55363058	q	IRX6	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg09849405	16	66612955	q	CMTM1	NA	3'UTR	island	3'UTR - island	NA	Not overlapping Differentially Methylated Region
cg08032924	16	66613096	q	CMTM2	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg16626067	16	66613266	q	CMTM2	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg06666025	16	66613278	q	CMTM2	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg07816687	16	67197186	q	HSF4	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg00748373	16	68676741	q	CDH3	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg16801887	16	86541480	q	FOXF1		-2653 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg16202470	17	8907213	p	NTN1		-17646 IGR	island	IGR - island	V\$AP2_Q6	Not overlapping Differentially Methylated Region
cg13291283	17	10101195	p	GAS7	NA	Body	island	Body - island	NA	Too far from other differentially methylated probes (>150bp)
cg25116216	17	10101581	p	GAS7		1stExon	island	1stExon - island	NA	Too far from other differentially methylated probes (>150bp)
cg08358166	17	18538271	p	TBC1D28		-571 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18686527	17	27044685	q	RAB34	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg21101720	17	27490509	q	ANKRD13B	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg14603098	17	32484259	q	ACCN1	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg15790037	17	37321490	q	ARL5C	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg13001868	17	43339223	q	C17orf46	NA	Body	island	Body - island	NA	Selected probes
cg04992638	17	43339328	q	C17orf46	NA	Body	island	Body - island	NA	Selected probes
cg03048083	17	43339497	q	LOC100133991	NA	Body	island	Body - island	NA	Selected probes
cg01627847	17	43339512	q	LOC100133991	NA	Body	island	Body - island	NA	Selected probes
cg08124910	17	43339515	q	LOC100133991	NA	Body	island	Body - island	NA	Selected probes
cg24542751	17	43339589	q	LOC100133991	NA	Body	island	Body - island	NA	Selected probes
cg02081266	17	59529618	q	TBX4		-4189 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg20275528	17	75369484	q	SEPT9	NA	5'UTR	island	5'UTR - island	NA	Too far from other differentially methylated probes (>150bp)
cg12783819	17	75369657	q	SEPT9	NA	5'UTR	island	5'UTR - island	NA	Too far from other differentially methylated probes (>150bp)
cg12865552	17	77721631	q	ENPP7		16749 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg11771234	18	908396	p	ADCYAP1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg22455914	18	7116977	p	LAMA1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg03117976	18	11149435	p	FAM38B	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg25958283	18	11752089	p	GNAL	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg21114773	18	12254452	p	CIDEA	NA	Body	island	Body - island	NA	Positivity in blood
cg12395205	18	12254469	p	CIDEA	NA	Body	island	Body - island	NA	Within centromere region
cg16727201	18	12254556	p	CIDEA	NA	Body	island	Body - island	NA	Positivity in blood
cg05700339	18	12254566	p	CIDEA	NA	Body	island	Body - island	NA	Within centromere region
cg27641522	18	53447560	q	MIR4529		301108 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg07519816	18	53447596	q	MIR4529		301144 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg27189087	18	56941246	q	RAX	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg12799689	18	67067893	q	DOK6	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg18596362	18	70210955	q	CBLN2	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg18448581	18	70534767	q	NETO1	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg05884032	18	76740088	q	SALL3	NA	TSS200	island	TSS200 - island	V\$MYOGNF1_01	Not overlapping Differentially Methylated Region
cg14007067	18	76740258	q	SALL3	NA	TSS200	island	TSS200 - island	NA	Positivity in blood
cg11616651	19	2251837	p	AMH	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg22963915	19	3785855	p	MATK	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg25936054	19	15090242	p	SLC1A6		29251 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg04704053	19	15090275	p	SLC1A6		29284 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg18652900	19	15580445	p	PGLYRP2	NA	Body	island	Body - island	NA	Positivity in blood
cg16206460	19	15580700	p	PGLYRP2	NA	Body	island	Body - island	NA	Positivity in blood
cg08607018	19	31842873	q	TSZH3		77022 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg25999442	19	34113398	q	CHST8	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg04332534	19	37096487	q	ZNF382	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg11957331	19	42828156	q	TMEM145	NA	Body	island	Body - island	V\$TAXCREB_02	Not overlapping Differentially Methylated Region
cg09489306	19	44203913	q	IRGC		-16301 IGR	island	IGR - island	NA	Not overlapping Differentially Methylated Region
cg15779837	19	48918116	q	GRIN2D	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg22049569	19	54466538	q	CACNG8	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg15708153	19	56879554	q	ZNF542	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg26612735	19	56988806	q	ZNF667	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg08063125	19	56989543	q	ZNF667	NA	TSS1500	island	TSS1500 - island		

cg18579862	19	58095595 q	ZIK1	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg13668618	19	58446745 q	ZNF418	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg12961842	19	58446758 q	ZNF418	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg12028548	20	590799 p	TCF15	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg07113642	20	2781262 p	CPXM1	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg14262681	20	21084420 p	KIZ		-22204 IGR	shore	IGR - shore	NA	Not overlapping Differentially Methylated Region
cg17368760	20	23029287 p	THBD	NA	1stExon	island	1stExon - island	NA	Selected probes
cg16339238	20	23029298 p	THBD	NA	1stExon	island	1stExon - island	NA	Selected probes
cg22388634	20	25058429 p	VSX1	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg14763548	20	25062447 p	VSX1	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg10615414	20	37353096 q	SLC32A1	NA	TSS200	island	TSS200 - island	NA	Selected probes
cg08313939	20	37353117 q	SLC32A1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg24454144	20	37353126 q	SLC32A1	NA	5'UTR	island	5'UTR - island	NA	Selected probes
cg11172693	20	37434229 q	PPP1R16B	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg24033330	20	39317034 q	MAFB	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg03403065	20	41818358 q	PTPRT	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region
cg10439765	20	44657838 q	SLC12A5	NA	1stExon	island	1stExon - island	NA	Positivity in blood
cg14060496	20	61638518 q	BHLHE23	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg26492446	20	61638574 q	BHLHE23	NA	TSS200	island	TSS200 - island	NA	Within telomer region
cg27501878	20	61638588 q	BHLHE23	NA	TSS1500	island	TSS1500 - island	NA	Within telomer region
cg15699267	20	61809557 q	MIR124-3	NA	TSS1500	island	TSS1500 - island	NA	Not overlapping Differentially Methylated Region
cg27357571	21	34398226 q	OLIG2	NA	TSS200	island	TSS200 - island	NA	Not overlapping Differentially Methylated Region
cg00495860	21	38065524 q	SIM2		-6467 IGR	island	IGR - island	NA	Positivity in blood
cg21697851	21	38076869 q	SIM2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg10682155	21	38077473 q	SIM2	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg24080247	21	38082613 q	SIM2	NA	Body	shore	Body - shore	NA	Positivity in blood
cg25446076	21	38083149 q	SIM2	NA	Body	shore	Body - shore	NA	Not overlapping Differentially Methylated Region
cg05206884	22	33454324 q	SYN3	NA	5'UTR	island	5'UTR - island	NA	Not overlapping Differentially Methylated Region
cg04198308	22	48971959 q	FAM19A5	NA	Body	island	Body - island	NA	Not overlapping Differentially Methylated Region
cg03957481	22	50986962 q	KLHDC7B	NA	1stExon	island	1stExon - island	NA	Not overlapping Differentially Methylated Region

Supplementary File 2C

Name	5'-->3' Sequence (for Bisulfite converted DNA)	Mg (mM)	Tm (°C)	length (bp)	Taq (unit)	Genomic_location_hg19	Encompassing probes from Illumina Infinium 450k.
Tag1	TCCCGCGAAATTAATACGAC						
Tag2	GCTGGAGCTCTGCAGCTA						
GRIA4-FW	TCCCGCGAAATTAATACGAC	1.5	62	106		chr11:105481255-105481360	cg15603568;cg23559689;cg00343633;cg03225817;cg04747226;cg07972135
GRIA4-RV	GCTGGAGCTCTGCAGCTA						
GRIA4-met-Probe	/5Alex647N/AACGCCGCGACCGCCACAC						
GRIA4-unm-Probe	/5Alex488N/CACCACAACCACCACACACA						
MSC-FW	TCCCGCGAAATTAATACGAC	1.5	58	122		chr8:72756000-72756121	cg25832771
MSC-RV	GCTGGAGCTCTGCAGCTA						
MSC-met-Probe	/5Alex647N/CGCATCCGAACACGCTCAC						
MSC-unm-Probe	/5Alex488N/ACACATCCAAACACACTCAC						
EYA4-FW	TCCCGCGAAATTAATACGAC	2	54	96		chr6:133562421-133562516	cg05085230; cg08712932; cg06132028; cg11942956; cg20787173; cg11664500; cg14287112; cg22871668;
EYA4-RV	GCTGGAGCTCTGCAGCTA						
EYA4-met-Probe	/5Alex647N/GACCGTTCCCGACTTCCGC						
EYA4-Unmet-Probe	/5Alex488N/CACTCCAACCATTCCCA						
MAP3K14-AS1-FW	TCCCGCGAAATTAATACGAC	2.5	50	99		chr17:43339469-43339567	cg17222164; cg03048083; cg01627847; cg08124910
MAP3K14-AS1-RV	GCTGGAGCTCTGCAGCTA						
MAP3K14-AS1-met-Probe	/5Alex647N/CGACGACATCACAAACACCGACG						
MAP3K14-AS1-unm-Probe	/5Alex488N/CAACAACATCACAAACACCAACA						
ITGA4-FW	TCCCGCGAAATTAATACGAC	2.5	50	76		chr2:182,322,231-182,322,306	cg06952671; cg21995919
ITGA4-RV	GCTGGAGCTCTGCAGCTA						
ITGA4-met-Probe	/5Alex647N/ACCCGCTAACGCCGAACACGCTA						
ITGA4-unm-Probe	/5Alex488N/ACCCACTAACACCAAACACACTA						

Name	Catalog #	Tm(°C)
BRAF V600E	dHsaCP2000027	55
KRAS G13D	dHsaMDV2510598	55
KRAS G12D	dHsaMDV2510596	55
KRAS G12A	dHsaMDV2510586	55
KRAS G12R	dHsaMDV2510590	55
KRAS G12V	dHsaMDV2510592	55
KRAS G12C	dHsaMDV2510584	55
KRAS Q61H	dHsaMDV2010131	55
MET CNV	dHsaCP2500321	60

Supplementary File 2D

Clinical data summary for the two cohorts of cfDNA donors involved in the study

Aging Cohort	N	(%)
Sex	25	(50)
	25	(50)
Age (years)	61	
	[40-65]	

mCRC cohort	N	(%)
Sex	Male 117	(64.3)
	Female 65	(35.7)
Age (years)	median 64	
	Range [33-85]	
CEA (ng/ml)	median 66.1	
	Range [0.8-20543]	
	<=5 27	(14.9)
	>5 154	(85.1)
# previous treatment lines	NA 1	(0.6)
	0 38	(20.9)
	1 19	(10.4)
	2 43	(23.6)
	3 37	(20.3)
RECIST (mm) (quartiles)	4+ 45	(24.7)
	[9-49.25[44	(24.3)
	[49.25-81.55[43	(23.8)
	[81.55-117[42	(23.2)
	[117-250] 45	(24.9)
Primary tumor resected	NA 7	(3.9)
	Yes 150	(82.9)
Metastatic lesions	No 31	(17.1)
	1 49	(26.9)
	2+ 128	(70.3)
Bulky Disease	NA 5	(2.7)
	No 122	(67)
	Yes 47	(25.8)
RAS	NA 13	(7.1)
	wild type 107	(58.8)
	mutated 75	(41.2)
BRAF	NA 0	(0)
	wild type 133	(73.1)
	mutated 13	(7.1)
	NA 36	(19.8)

Supplementary File 2E

Case ID	Gender	DOB	Age	gE/ml (LINE1)	Plasma Date	DATE CEA	CEA	DELTA T CEA/BLOOD BRAW	TREATMENT	Start Treatment	Last administration (before Plasma Collecting)	DELTA BASELINE- PLASMA	PCR	TC Scan Date	DELTA BASELINE	TUMOR BURDEN RECIST (mm)	BULKY DISEASE T IN SITU	N metastatic sites	LIVER	LUNG	NODES	PERITONEUM	BONE	OTHER SITES	KRAS	NRAS	KRAS+NRAS	BRAF					
CRC_Monitoring_1	F	1-Jan-43	69.0	52861.4	9-Dec-11	9-Dec-11	100	0	Dacarbazine	10-Nov-11	30-Nov-11	0	-9	3	10-Nov-11	-29	158	0	3	1	1	0	1	0	1	1	0	1	0				
CRC_Monitoring_2	F	17-Oct-34	76.1	60603.1	11-Sep-10	10-Nov-10	229.2	-1	PmAb	15-Sep-10	10-Nov-10	0	-92	-1	2	16-Nov-10	5	109	0	2	0	1	0	1	1	0	0	0	0				
CRC_Monitoring_3	F	4-Aug-67	47.2	26504.8	15-Oct-14	17-Sep-14	765.6	-28	MEK162	1-Oct-14	1-Oct-14	0	-14	2	17-Nov-14	33	115	1	0	2	1	0	1	0	0	0	0	0	0				
CRC_Monitoring_4	M	5-Apr-45	69.4	35085.2	5-Aug-14	2-Jul-14	17.7	-34	no treatment			1		2	16-Jul-14	-20	104	1	0	2	0	1	1	0	0	0	0	1	1	0			
CRC_Monitoring_5	F	25-Dec-66	48.3	35851.8	8-Apr-15	13-Apr-15	387.2	5	HERACLES	14-Apr-15	14-Apr-15	1	6	4	8-Apr-15	0	124	0	0	3	1	1	1	0	0	0	0	0	0				
CRC_Monitoring_6	M	25-Dec-44	64.8	30699.0	9-Sep-09	18-Sep-09	45.5	9	no treatment			1		4	16-Sep-09	7	193	0	0	4	1	0	1	0	0	2		0	0				
CRC_Monitoring_7	F	17-Aug-54	55.8	30438.3	4-May-10	3-May-10	3	-1	no treatment			1		2	4-Jun-10	31	65	1	0	3	1	0	1	0	0	1	0	0	0				
CRC_Monitoring_8	M	7-Aug-29	83.0	207913.5	29-Jun-12	28-Jun-12	1738	-1	Folfinr+Beva	29-Jun-12	29-Jun-12	1	0	2	7-Jun-12	-22	109	0	0	1	1	0	0	0	0	0	0	0	0				
CRC_Monitoring_9	M	31-Jul-47	64.0	88959.6	8-Jul-11	20-Jun-11	119.8	-18	Folfinr	20-Jun-11	20-Jun-11	0	-18	3	11-Jul-11	3	192	1	0	2	1	0	1	0	0	0	0	1	1	0			
CRC_Monitoring_10	M	28-Jan-46	68.8	204675.3	6-Nov-14	10-Nov-14	6.4	4	Folfinr	20-Oct-14	20-Oct-14	0	-17	0	7-Nov-14	1	86	0	0	3	1	0	1	0	1	0	0	0	1	0			
CRC_Monitoring_11	M	6-Jul-58	56.9	196187.1	18-May-15	7-May-15	32.9	-11	ELO	18-Mar-15	18-Mar-15	0	-61	1	7-May-15	-11	31	0	0	1	1	0	0	0	0	0	0	0	0	0			
CRC_Monitoring_12	M	9-Sep-44	71.2	84931.2	23-Oct-15	23-Oct-15	9.8	0	Folfinr+Beva	9-Nov-15	2-Mar-15	1	-235	1	27-Oct-15	4	28	0	0	1	0	0	0	0	0	1	1	0	1	0			
CRC_Monitoring_13	M	23-Oct-60	51.0	448292.9	3-Oct-11	19-Sep-11	835.5	-14	GA201	10-Oct-11	15-Sep-11	0	-18	1	19-Sep-11	-14	122	1	1	2	0	1	1	0	0	0	0	0	0	0			
CRC_Monitoring_14	F	20-Mar-43	68.2	19396.6	25-May-11	25-May-11	60.5	0	PmAb	25-May-11	24-May-11	0	-1	2	15-Apr-11	-40	43	0	0	4	1	1	0	0	0	1	0	0	1	0			
CRC_Monitoring_15	M	17-Apr-32	80.8	181456.6	7-Jan-13	7-Jan-13	2.6	0	PmAb	5-Dec-12	2-Sep-15	491.2	-18	3	28-Jan-13	21	35	0	0	2	0	1	0	0	0	0	0	0	0	0			
CRC_Monitoring_16	M	18-Feb-42	73.4	215947.9	2-Jul-15	8-Jul-15	1.1	0	PmAb+Tramet	16-Jul-15	16-Jul-15	1	14	3	9-Jul-15	7	12	0	0	2	0	1	0	0	0	0	0	0	0	1	0		
CRC_Monitoring_17	M	26-Nov-43	69.6	10659.3	24-Jun-10	24-Jun-10	21	0	no treatment			1		1	3-May-10	-52	117	0	0	2	1	0	0	1	0	0	0	0	0	1	0		
CRC_Monitoring_18	F	4-Oct-60	54.5	154841.2	8-Apr-15	8-Apr-15	9762	0	Irinotecan	1-Apr-15	1-Apr-15	0	-7	3	26-Mar-15	-13	226	1	1	3	1	1	1	0	0	0	0	0	0	0	0		
CRC_Monitoring_19	M	30-Nov-37	72.5	120155.3	25-May-10	23-Jun-10	8.7	29	Irinotecan	22-May-10	22-May-10	0	-3	0	4-May-10	-21	117	0	1	1	1	0	0	0	0	0	0	0	0	0	0		
CRC_Monitoring_20	M	3-Jul-62	49.6	47477.6	6-Feb-12	5-Dec-11	228.6	-63	Pre-Surgery - No treatment			0		1	1-Feb-12	-5	83	0	1	1	1	0	0	0	0	0	0	0	0	0	0		
CRC_Monitoring_21	M	25-May-39	74.5	131904.1	18-Nov-13	18-Nov-13	858.9	0	no treatment			1		3	18-Nov-13	0		1	1	2	1	1	0	0	0	0	1	1	0	1	0		
CRC_Monitoring_22	M	21-Jul-34	76.0	48021.2	20-Jul-10	19-Jul-10	27.7	-1	Follo	14-May-10	19-Jul-10	0	-1	0	9-Aug-10	20	113	1	0	1	1	0	0	0	0	0	0	0	0	0	0		
CRC_Monitoring_23	F	11-Feb-41	70.0	95896.7	19-Jan-11	18-Jan-11	89.3	-1	Folfinr	15-Sep-10	18-Jan-11	0	-1	1	19-Apr-11	90	44	0	0	2	0	0	0	1	0	1	1	1	0	1	0		
CRC_Monitoring_24	M	24-Dec-58	56.8	21320.9	18-Sep-15	2-Sep-15	491.2	-16	PmAb+Tramet	4-Aug-15	4-Sep-15	0	-14	3	15-Oct-15	27	87	1	0	3	0	1	1	0	0	1	0	0	0	1	0		
CRC_Monitoring_25	M	25-May-49	61.1	1294369.3	23-Jun-10	23-Jun-10	107.8	0	Follo	23-Jun-10	23-Jun-10	1	0	0	4-Jun-10	-19	58	0	1	3	1	0	0	0	0	0	0	0	0	0	0		
CRC_Monitoring_26	M	11-Apr-41	69.6	157496.2	19-Oct-10	28-Sep-10	1.7	-21	Follo+Cmab	6-Aug-10	6-Aug-10	0	-21	0	27-Oct-10	8	84	0	1	1	1	0	0	0	0	0	0	0	0	0	0		
CRC_Monitoring_27	F	13-Nov-34	79.8	272173.8	4-Aug-14	23-Jun-14	113.6	-42	Folfinr	14-May-14	22-Jul-14	0	-13	2	30-Jun-14	-35	84	0	1	3	1	1	0	0	1	0	1	1	0	1	0		
CRC_Monitoring_28	M	11-May-51	63.6	409422.1	20-Nov-14	20-Nov-14	7.3	0	Pre-Surgery - No treatment			1		1	21-Oct-14	-30	42	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_29	F	28-Apr-40	74.2	288828.3	24-Jun-14	25-Jun-14	2.7	1	Follo	20-May-14	23-Jun-14	0	-1	0	22-May-14	-33	167	0	0	3	0	0	1	1	0	1	0	0	0	1	0		
CRC_Monitoring_30	M	28-Nov-45	68.7	21913.7	1-Aug-14	24-Jun-14	67.4	-38	Folfinr	16-Jul-14	16-Jul-14	0	-16	1	30-Jun-14	-32	161	0	0	3	1	0	1	0	0	1	1	0	1	0	1	0	
CRC_Monitoring_31	F	12-Apr-64	49.8	214073.7	21-Jan-14	21-Jan-14	473.5	0	no treatment			1		3	15-Jan-14	-6		0	3	1	0	1	0	1	0	1	0	0	0	1	0		
CRC_Monitoring_32	F	30-Jun-45	69.7	164095.6	11-Mar-15	8-Jan-15	5.5	-62	Pre-Surgery - No treatment			1		1	11-Mar-15	0	46	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	
CRC_Monitoring_33	M	16-Nov-35	78.7	176952.6	26-Jun-14	26-Jun-14	278.2	0	Pre-Surgery - No treatment			0		1	7-Apr-14	-80	43	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_34	M	1-May-28	82.7	77812.2	21-Dec-14	24-Dec-10	4.3	3	Folfinr	11-Dec-10	11-Dec-10	0	-10	1	16-Dec-10	5	57	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	
CRC_Monitoring_35	M	26-May-42	72.3	155034.4	2-Sep-14	8-Jul-14	24.2	-57	PmAb	11-Sep-14	9-Jul-14	0	-56	2	9-Sep-14	6	81	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_36	F	19-Jul-40	73.8	485302.7	8-Apr-14	17-Mar-14	4189	-22	Follo	23-Jan-14	7-Apr-14	0	-1	0	15-May-14	-37	175	1	1	2	1	0	1	0	0	1	0	1	0	1	0	0	
CRC_Monitoring_37	F	20-Dec-40	69.8	3718000.8	14-Sep-10	14-Sep-10	48.5	0	Mitomycin+5FU	14-Sep-10	4-Aug-10	0	-41	2	9-Aug-10	-36	61	0	0	2	1	0	0	0	0	0	0	1	1	0	1	0	
CRC_Monitoring_38	F	19-Jan-42	72.7	517187.6	10-Sep-14	26-Aug-14	4.8	-15	no treatment			1		0	10-Sep-14	0	19	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_39	M	25-May-60	50.2	33734.4	28-Jul-10	13-Aug-10	3.3	16	PmAb	9-Apr-10	1-Jul-10	0	-27	2	8-Jul-10	-20	18	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_40	M	29-Apr-44	70.0	10562.6	14-Apr-14	14-Apr-14	17.2	0	no treatment			1		3	2-Apr-14	-12	72	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_41	M	20-Apr-48	67.0	6878.7	23-Mar-15	23-Mar-15	1.7	0	Pre-Surgery - No treatment			1		1	23-Mar-15	0	75	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_42	M	30-Jun-40	72.5	25247.4	11-Dec-12	11-Dec-12	2	0	PmAb	14-Aug-12	27-Nov-12	0	-14	2	14-Dec-12	3	99	0	0	2	1	0	1	0	0	0	1	1	0	1	0	0	
CRC_Monitoring_43	M	26-Oct-36	79.0	167832.7	15-Oct-15	13-Oct-15	2	-2	Pre-Surgery - No treatment			1		0	15-Oct-15	0	25	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_44	M	20-Apr-59	59.3	103753.4	5-Aug-14	5-Aug-14	8.6	0	no treatment			1		1	11-Sep-14	37	81	1	1	4	1	0	0	0	0	1	1	0	0	0	1	0	
CRC_Monitoring_45	M	16-Jul-74	40.3	818211.6	5-Nov-14	5-Nov-14	1267	0	Pre-Surgery - No treatment			1		1	6-Nov-14	1	75	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_46	M	1-Jun-29	81.0	96154.5	11-May-10	11-May-10	7.5	0	Follo	9-May-10	9-May-10	0	-2	2	22-Apr-10	-19	41	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	
CRC_Monitoring_47	M	21-Dec-68	46.5	28029.4	10-Jun-15	10-Jun-15	31.4	0	no treatment			1		4	16-May-15	-25	110	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
CRC_Monitoring_48	M	16-Jul-35	75.3	8874.5	12-Oct-10	9-Nov-10	27	28	Folfinr	3-Sep-10	24-Sep-10	0	-18	2	20-Jul-10	-84	10	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	
CRC_Monitoring_49	M	29-Nov-41	73.5	39603.6	8-May-15	20-Apr-15	12.1	-18	Follo	8-May-15	20-Apr-15	0	-18	0	19-Jun-15	42	32	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0
CRC_Monitoring_50	F	11-Jul-56	58.1	83437.2	23-Jul-14	22-Jul-14	250	-1	Pre-Surgery - No treatment			1		0	23-Jul-14	0	55	0	1	1	1	0	0	0	0	0	0	1	1	0	1	0	
CRC_Monitoring_51	M	23-Sep-47	67.7	41904.5																													

CRC_Monitoring_82	F	24-Oct-63	51.3	663475.4	9-Feb-15	9-Feb-15	3.3	0	PmAb+Folfox	13-Jan-15	27-Jan-15	0	-13	0	9-Jan-15	-31	90	0	1	3	1	0	1	0	0	1	0	0	0	0
CRC_Monitoring_83	M	15-Sep-63	49.6	58741.5	28-Mar-13	11-Feb-13	120.8	-45	CmAb+irinotecan	28-Mar-13	9-Mar-13	0	-19	0	2-May-13	35	60	0	0	2	1	0	0	0	0	0	0	0	0	0
CRC_Monitoring_84	F	31-Jul-37	76.8	192324.6	18-Apr-14	24-Mar-14	7.5	-25	CmAb+Folfox	3-Jan-14	8-Apr-14	0	-10	0	12-May-14	24	149	0	0	2	1	1	0	0	0	0	0	0	0	0
CRC_Monitoring_85	M	18-Feb-38	76.0	37876.5	30-Jan-12	30-Jan-12	63.5	0	Dacarbazine	9-Jan-12	9-Jan-12	0	-21	4	17-Dec-11	-44	107.3	0	0	4	1	1	1	0	0	1	1	0	0	
CRC_Monitoring_86	M	9-May-54	57.6	23825.4	11-Nov-11	15-Nov-11	580.1	0	Dacarbazine	15-Nov-11	15-Nov-11	0	-30	1	14-Nov-11	3	153	0	0	3	1	1	1	0	0	0	0	0	0	
CRC_Monitoring_87	F	27-Nov-67	44.3	18227.6	15-Mar-12	15-Mar-12	1.1	1	0	15-Mar-12	15-Mar-12	0	0	6	15-Mar-12	0	38.4	0	0	1	0	0	1	0	0	0	0	0	0	
CRC_Monitoring_88	M	4-Nov-56	54.9	88515.9	31-Aug-11	25-Aug-11	11.4	-6	Dacarbazine	29-Aug-11	1-Jul-11	1	-61	4	26-Aug-11	-5	98.3	0	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_89	F	23-Dec-35	75.7	48028.9	9-Aug-11	8-Aug-11	66.1	-1	Dacarbazine	15-Jun-11	8-Aug-11	1	-55	3	8-Aug-11	-1	75.9	0	0	2	1	0	1	0	0	0	1	1	0	
CRC_Monitoring_90	M	27-Nov-44	67.2	31252.6	16-Jan-12	16-Jan-12	12.3	0	Dacarbazine	23-Jan-12	15-Oct-11	1	-93	3	26-Jan-12	10	97.1	0	0	2	1	0	1	0	0	0	0	0	0	
CRC_Monitoring_91	M	6-Apr-58	53.8	12879.9	9-Jan-12	9-Jan-12	161.9	0	Dacarbazine	12-Jan-12	15-Nov-11	1	-55	4	11-Jan-12	2	79.3	0	0	4	1	1	1	0	0	1	0	0	0	
CRC_Monitoring_92	F	14-Jul-36	75.1	44150.3	28-Jul-11	22-Aug-11	102.5	25	Dacarbazine	29-Jul-11	28-Jun-11	0	-30	3	28-Jul-11	0	64	0	0	1	0	0	1	0	0	0	1	1	0	
CRC_Monitoring_93	M	5-Jan-38	74.0	16070.5	5-Dec-11	5-Dec-11	101.1	0	Dacarbazine	4-Oct-11	13-Nov-11	0	-22	4	3-Oct-11	-63	64	1	0	2	0	1	1	0	0	0	1	1	0	
CRC_Monitoring_94	M	6-Sep-52	58.9	14936.5	15-Jul-11	15-Jul-11	30.3	0	Dacarbazine	15-Jul-11	10-Jun-11	1	-35	4	16-Jun-11	-29	39.5	0	0	2	1	1	0	0	0	0	1	1	0	
CRC_Monitoring_95	F	19-May-55	56.1	168268.6	9-Jun-11	9-Jun-11	443.8	0	Dacarbazine	9-Feb-11	10-Jun-11	1	-120	4	10-Jun-11	1	29	1	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_96	M	1-Jun-75	59.5	74046.0	5-Dec-11	5-Dec-11	2057	0	Dacarbazine	29-Oct-11	9-Dec-11	1	-38	2	9-Dec-11	0	93.2	1	0	1	1	0	0	0	0	0	0	0	0	
CRC_Monitoring_97	M	30-Sep-41	70.1	124077.1	24-Oct-11	24-Oct-11	4.2	0	Dacarbazine	25-Oct-11	15-Aug-11	1	-70	4	24-Oct-11	0	63.1	1	0	2	1	1	0	0	0	0	1	1	0	
CRC_Monitoring_98	F	5-Feb-52	59.9	20085.5	29-Nov-11	28-Nov-11	299.6	-1	Dacarbazine	3-Oct-11	14-Nov-11	1	-57	4	14-Nov-11	-15	69	0	0	1	0	1	0	0	0	0	0	0	0	
CRC_Monitoring_99	M	10-Apr-52	59.2	133205.0	8-Jun-11	8-Jun-11	9.7	0	Dacarbazine	9-Jun-11	15-Feb-11	0	-113	2	8-Jun-11	0	128	0	0	3	1	1	1	0	0	0	1	1	0	
CRC_Monitoring_100	F	15-Jun-44	67.4	278709.5	5-Oct-11	3-Oct-11	868.5	-2	Dacarbazine	12-Sep-11	4-Oct-11	0	-23	3	4-Oct-11	-1	53.3	0	0	2	1	1	0	0	0	0	1	1	0	
CRC_Monitoring_101	F	10-Apr-39	72.8	255619.6	23-Jan-12	23-Jan-12	26.1	0	Dacarbazine	24-Jan-12	16-Dec-11	0	-1	3	27-Dec-11	-27	181	1	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_102	M	25-Aug-34	77.1	108563.7	28-Sep-11	28-Sep-11	968.8	0	Dacarbazine	1-Oct-11	15-Jun-11	1	-108	5	29-Sep-11	0	125.6	1	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_103	M	24-Jun-49	62.7	80164.5	14-Feb-12	14-Feb-12	241.5	0	Dacarbazine	14-Feb-12	13-Jan-12	1	-32	7	9-Feb-12	-5	191.9	0	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_104	F	21-Apr-63	48.8	29126.1	10-Feb-12	18-Feb-12	185.5	0	Dacarbazine	27-Feb-12	1-Feb-12	1	-58	4	1-Feb-12	-9	160	0	0	4	1	1	1	0	0	1	0	0	0	
CRC_Monitoring_105	M	24-Aug-47	64.6	10285.6	14-Mar-12	14-Mar-12	12.7	0	Dacarbazine	17-Mar-12	15-Feb-12	0	-28	3	26-Mar-12	0	107.9	0	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_106	M	15-Jan-46	66.2	2046.3	12-Mar-12	12-Mar-12	155.4	0	Dacarbazine	20-Feb-12	20-Feb-12	0	-21	5	17-Feb-12	-24	112.8	1	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_107	M	14-May-36	75.4	28040.5	5-Sep-11	5-Sep-11	60.2	0	Dacarbazine	6-Sep-11	11-Aug-11	0	-25	6	11-Aug-11	-25	112.6	0	0	2	0	1	0	0	0	1	1	1	0	
CRC_Monitoring_108	M	23-Apr-53	58.4	12029.2	19-Aug-11	19-Aug-11	10.8	0	Dacarbazine	14-Jun-11	26-Jul-11	0	-24	2	12-Aug-11	-7	24	0	0	3	1	0	1	1	0	0	1	1	0	
CRC_Monitoring_109	M	15-Mar-66	45.9	3700.3	30-Jan-12	30-Jan-12	231.9	0	Dacarbazine	31-Jan-12	19-Dec-11	1	-42	2	23-Jan-12	-7	97.9	1	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_110	M	23-Apr-75	36.9	12044.0	20-Feb-12	20-Feb-12	569	0	Dacarbazine	21-Feb-12	15-Dec-11	1	-67	6	6-Feb-12	-14	174	1	0	3	1	0	1	0	0	1	1	0	0	
CRC_Monitoring_111	M	15-Oct-54	57.0	28588.4	19-Sep-11	25-Aug-11	3.3	-25	Dacarbazine	29-Aug-11	29-Aug-11	0	-21	2	4-Aug-11	-46	117.6	0	0	2	1	0	1	0	0	0	1	1	0	
CRC_Monitoring_112	M	27-Sep-58	53.4	32088.4	26-Jan-12	25-Jan-12	78	-1	Dacarbazine	27-Jan-12	5-Dec-11	0	-52	5	26-Jan-12	0	55.5	1	0	2	1	1	0	0	0	0	1	1	0	
CRC_Monitoring_113	F	16-Nov-41	70.1	9732.7	29-Nov-11	28-Nov-11	2930	-1	Dacarbazine	29-Nov-11	7-Nov-11	0	-22	3	7-Nov-11	-22	65.6	0	0	2	1	1	0	0	0	0	0	0	0	
CRC_Monitoring_114	M	30-Jan-39	72.7	27679.7	13-Sep-11	22-Aug-11	1.9	-22	Dacarbazine	11-Jul-11	23-Aug-11	0	-21	4	9-Sep-11	-4	65	0	0	1	0	1	0	0	0	0	1	1	0	
CRC_Monitoring_115	F	9-Nov-69	41.9	266432.4	12-Sep-11	19-Aug-11	991.4	-24	Dacarbazine	28-Aug-11	23-Aug-11	0	-20	2		-28											1	1	0	
CRC_Monitoring_116	M	1-May-52	59.4	13431.5	2-Sep-11	19-Aug-11	45.2	-14	Dacarbazine	20-Jun-11	1-Aug-11	0	-32	3	5-Aug-11	-4	52.3	0	0	2	0	1	0	1	0	0	0	1	1	0
CRC_Monitoring_117	M	18-Aug-71	40.4	17440.7	17-Jan-12	17-Jan-12	44.8	0	Dacarbazine	23-Jan-12	15-Oct-11	1	-94	3	4-Jan-12	-13	70.3	0	0	2	1	0	1	0	0	0	1	1	0	
CRC_Monitoring_118	M	17-Jun-57	54.6	1110.9	24-Jan-12	24-Jan-12	7.1	0	Dacarbazine	26-Jan-12	15-Dec-11	1	-40	5	26-Jan-12	2	55.5	0	0	1	0	0	0	1	0	0	1	1	0	0
CRC_Monitoring_119	M	15-Jul-30	81.0	350586.4	13-Jun-11	10-Jun-11	167.8	-3	Dacarbazine	11-Jun-11	11-Jun-11	0	-2	3	10-Jun-11	-3	113	0	0	2	0	1	1	0	0	0	0	1	1	0
CRC_Monitoring_120	M	6-Jan-35	76.9	51240.2	7-Nov-11	3-Nov-11	1600	-4	Dacarbazine	4-Nov-11	4-Nov-11	0	-3	4	3-Nov-11	-4	53.7	0	0	1	1	0	0	0	0	0	0	0	0	0
CRC_Monitoring_121	M	17-Jun-42	69.6	106424.6	23-Jan-12	23-Jan-12	69.3	0	Dacarbazine	24-Jan-12	12-Dec-11	1	-42	4	19-Jan-12	-4	82.1	0	0	1	1	0	0	0	0	0	0	0	0	0
CRC_Monitoring_122	M	25-Feb-37	74.9	83442.6	18-Dec-11	12-Dec-11	282.2	0	Dacarbazine	13-Dec-11	29-Dec-11	0	-2	3	29-Dec-11	-16	109.6	0	0	2	1	0	0	0	0	1	0	0	0	0
CRC_Monitoring_123	M	9-Oct-37	74.2	925907.7	14-Dec-11	18-Dec-11	34.1	4	Dacarbazine	15-Dec-11	10-Oct-11	0	-60	2	14-Dec-11	0	198	1	0	2	1	0	1	0	0	0	1	1	0	
CRC_Monitoring_124	F	26-Nov-53	57.7	124284.9	8-Aug-11	4-Aug-11	82.9	-4	Dacarbazine	6-Aug-11	6-Aug-11	0	-2	4	5-Aug-11	-3	40.3	1	0	1	0	1	0	0	0	0	0	0	0	0
CRC_Monitoring_125	F	11-Nov-40	70.7	152843.3	4-Jul-11	4-Jul-11	151.7	0	Dacarbazine	15-Mar-11	1-Jul-11	0	-111	4	1-Jul-11	-3	40	1	0	0	2	1	1	0	0	0	0	0	0	0
CRC_Monitoring_126	M	29-May-48	63.5	10694.6	7-Nov-11	4-Nov-11	4.4	-3	Dacarbazine	8-Nov-11	7-Oct-11	1	-31	3	7-Nov-11	0	62.6	0	0	2	0	1	0	1	0	0	0	1	1	0
CRC_Monitoring_127	M	9-Jun-38	73.5	39522.9	11-Nov-11	11-Nov-11	211.5	0	Dacarbazine	15-Oct-11	15-Oct-11	0	-27	2	14-Nov-11	3	91	0	0	2	1	0	1	0	0	0	1	1	0	0
CRC_Monitoring_128	F	19-Feb-44	67.6	325832.1	26-Sep-11	26-Sep-11	2581	0	Dacarbazine	26-Sep-11	15-Aug-11	1	-42	4	10-Nov-11	-45	30	1	0	1	0	1	0	0	0	0	0	0	0	0
CRC_Monitoring_129	M	20-Oct-45	65.8	28485.4	20-Jul-11	18-Jul-11	246.4	-2	Dacarbazine	20-Jul-11	15-Jun-11	1	-35	6	19-Jul-11	-1	113.3	0	0	3	1	1	0	0	0	1	0	0	0	0
CRC_Monitoring_130	F	5-May-71	40.8	2361.0	10-Feb-12	10-Feb-12	18.6	0	Dacarbazine	11-Feb-12	2-Dec-11	0	-70	4	10-Feb-12	0	49	0	0	2	0	1	0	0	0	0	1	1	0	0
CRC_Monitoring_131	M	9-Sep-42	49.8	19482.1	1-Sep-11	30-Aug-11	79.9	-3	Dacarbazine	6-Aug-11	4-Aug-11	0	-2	6	19-Aug-11	-23	6	0	0	2	1	0	0	0	0	0	0	0	0	0
CRC_Monitoring_132	M	26-Aug-36	74.7	9992.4	3-May-11	4-May-11	5.3	1	PmAb	4-Apr-11	5-Apr-11	0	-29	2	4-Apr-11	-29	50	0	0	1	1	1	0	0	0	0	0	0	0	0
CRC_Monitoring_133	F	20-Sep-67	45.8	4188490	11-Jul-13	11-Jul-13	3321	0	PmAb	12-Jul-13	17-Apr-13	1	-85	4	27-Jun-13	-14	65	1	1	1	1									

CRC_Monitoring_174	M	17-Mar-44	70.0	9661.0	10-Feb-14	24-Oct-13	49.8	-109	no treatment	10-Feb-14			1		0	29-Oct-13	-104	47		0							1	1	0
CRC_Monitoring_175	F	13-Aug-47	66.8	341927.0	29-Apr-14	29-Apr-14	79	0	no treatment	29-Apr-14			1		0	4-Apr-14	-25	182		1	4	1	1		1		1	1	0
CRC_Monitoring_176	F	25-Jun-68	45.9	7014008.0	12-May-14	26-May-14	518.3	14	no treatment	12-May-14			1		0	14-Apr-14	-28	70	0	1	2	1		1			1	1	0
CRC_Monitoring_177	M	22-May-37	77.0	29728.0	13-May-14	13-May-14	455.9	0	no treatment	13-May-14			1		0	17-Apr-14	-26	110	0	0	4		1	1	1		1	1	0
CRC_Monitoring_178	F	5-Mar-52	61.4	95599.0	2-Jul-13	2-Jul-13	527	0	no treatment	2-Jul-13			1		0	20-Jun-13	-12	70		1							1	1	0
CRC_Monitoring_179	M	25-Jan-59	54.5	21720.0	3-Jul-13	23-May-13	1.8	-41	no treatment	3-Jul-13			1		0	20-May-13	-44	50		0							1	1	0
CRC_Monitoring_180	F	8-Apr-45	68.3	69420.0	15-Jul-13	15-Jul-13	28.9	0	no treatment	15-Jul-13			1		0	23-May-13	-53	70	0	0	1	1					1	1	0
CRC_Monitoring_181	M	12-Apr-52	64.0	13540.4	12-Apr-16				Folfox+Beva	3-May-16			1		0	11-Apr-16	-1	35	0	0	3	1		1	1			1	0
CRC_Monitoring_182	M	11-Dec-40	75.5	5980.8	19-May-16	4-May-16	2.8	-15	Folfox+Beva	5-May-16			0		-14	0	4-May-16	-15	56	0	0	2	1	1				1	0

[illegible]

Supplementary File 2H

Association between clinico-pathological features and circulating DNA methylation

	Median	pvalue
Sex	Female 35.60 Male 11.80	0.029
Age (years)	[33-54.7[23.25 [54.7-62.9[12.80 [62.9-72.7[12.40 [72.7-85.2] 17.85	<i>ns</i>
CEA (ng/ml)	<=5 2.80 >5 18.60	<i>ns</i>
RECIST (mm) (quartiles)	[9-49.25[3.15 [49.25-81.55[17.10 [81.55-117[18.10 [117-250] 33.80	<i>ref.</i> 0.06 <i>ns</i> 0.006
GE/ml (quartiles)	[1110-19613[2.40 [19613-40107[10.10 [40107-137394[34.20 [137394-9259908] 69.95	<i>ref.</i> <i>ns</i> <0.001 <0.001
Primary tumor intact	No 12.45 Yes 53.20	0.002
Metastatic lesions	1 5.50 2+ 22.45	0.023
Bulky disease	No 11.85 Yes 39.00	0.012
RAS	wild type 15.60 mutated 17.80	<i>ns</i>
BRAF	wild type 15.70 mutated 47.40	<i>ns</i>
Under treatment	No 18.35 Yes 14.20	<i>ns</i>

Supplementary File 21

Case_ID	Collection_Date	Days	Average marker>1% in BL	Mn-M0	Best_CH3_chan ge	Date CT Scan	Days to plasma baseline	Days to closest blood draw	SUM target lesions	PD x new lesions (y/n)	RECIST_C hange	BEST_R ECIST	CEA	PFS	Best_response (0:PD; 1:SD; 2:PR)	MGMT Methylation at baseline
CRC_Monitoring_142	28-Dec-12	0	34.5	0.0		4-Dec-12	-24	-24	141	0			164	2.80	1	42.9
CRC_Monitoring_142	9-Jan-13	12	19.7	-14.8	x								107.3	2.80	1	42.9
CRC_Monitoring_142	25-Jan-13	28	50.3	15.7		8-Feb-13	42	14	137		-2.8	X	197.4	2.80	1	42.9
CRC_Monitoring_142	20-Feb-13	54	47.6	13.0									150.4	2.80	1	42.9
CRC_Monitoring_142	1-Mar-13	63	38.0	3.4		22-Mar-13	84	21	152	y	7.8		209.4	2.80	1	42.9
CRC_Monitoring_143	25-Jan-13	0	51.4	0.0		23-Jan-13	-2	-2	117	n			44.5	2.77	1	44.3
CRC_Monitoring_143	14-Feb-13	20	3.8	-47.7									30.3	2.77	1	44.3
CRC_Monitoring_143	20-Feb-13	26	0.2	-51.3	x								32.5	2.77	1	44.3
CRC_Monitoring_143	4-Mar-13	38	5.8	-45.7		8-Mar-13	42	4	97		-17.1	X	48.5	2.77	1	44.3
CRC_Monitoring_143	27-Mar-13	61	54.2	2.8									92.1	2.77	1	44.3
CRC_Monitoring_143	23-Apr-13	88	68.0	16.6		19-Apr-13	84	-4	119		1.7		115.6	2.77	1	44.3
CRC_Monitoring_144	23-Aug-13	0	2.3	0.0		6-Aug-13	-17	-17	97	0			13.3	4.30	1	14.6
CRC_Monitoring_144	23-Oct-13	61	13.6	11.4	x	10-Oct-13	48	-13	95		-2.1	X	29.3	4.30	1	14.6
CRC_Monitoring_144	18-Dec-13	117	62.7	60.4		20-Nov-13	89	-28	102		5.2		22	4.30	1	14.6
CRC_Monitoring_144	27-Jan-14	157	72.7	70.4		3-Jan-14	133	-24	99	y	2.1		25.7	4.30	1	14.6
CRC_Monitoring_145	19-Dec-13	0	2.0	0.0		20-Dec-13	1	1	84	0			80	1.37	0	0.0
CRC_Monitoring_145	22-Jan-14	34	1.2	-0.7	x	3-Feb-14	46	12	100	y	19	X	117.9	1.37	0	0.0
CRC_Monitoring_146	5-Jul-13	0	15.6	0.0		10-Jul-13	5	5	126	n			147.6	2.77	1	0.0
CRC_Monitoring_146	8-Aug-13	34	40.5	24.9	x	21-Aug-13	47	13	146		15.9	X	238.1	2.77	1	0.0
CRC_Monitoring_146	5-Sep-13	62	52.1	36.6									309.1	2.77	1	0.0
CRC_Monitoring_146	12-Sep-13	69	84.0	68.4									334.5	2.77	1	0.0
CRC_Monitoring_146	10-Oct-13	97	61.7	46.1		3-Oct-13	90	-7	165		31		592.3	2.77	1	0.0
CRC_Monitoring_147	3-Sep-13	0	29.3	0.0		3-Aug-13	-31	-31	133	0			112.9	2.87	1	4.0
CRC_Monitoring_147	2-Oct-13	29	2.2	-27.2	x	15-Oct-13	42	13	127		-4.5		52.3	2.87	1	4.0
CRC_Monitoring_147	30-Oct-13	57	2.2	-27.1		29-Nov-13	87	30	105	y	-21.1	X	16.6	2.87	1	4.0
CRC_Monitoring_148	21-Jan-13	0	77.8	0.0		21-Jan-13	0	0	135	n			3389	1.37	0	61.9
CRC_Monitoring_148	11-Feb-13	21	88.2	10.5	x	11-Mar-13	49	28	163	y	20.7	X	3440	1.37	0	61.9
CRC_Monitoring_149	14-Jan-13	0	12.8	0.0		21-Dec-12	-24	-24	167	n			1866	1.63	0	9.7
CRC_Monitoring_149	11-Feb-13	28	27.3	14.6	x								2148	1.63	0	9.7
CRC_Monitoring_149	8-Mar-13	53	49.3	36.6		6-Mar-13	51	-2	194	y	16.2	X	2446	1.63	0	9.7
CRC_Monitoring_151	6-May-13	0	72.7	0.0		6-May-13	0	0	53	0			11	1.30	0	69.3
CRC_Monitoring_151	7-Jun-13	32	84.5	11.8	x	21-Jun-13	46	14	92		73.6	X	21.6	1.30	0	69.3
CRC_Monitoring_151	5-Jul-13	60	88.9	16.2									52.7	1.30	0	69.3
CRC_Monitoring_152	14-Mar-13	0	37.4	0.0		11-Mar-13	-3	-3	34	n			19.2	2.77	1	0.0
CRC_Monitoring_152	26-Mar-13	12	13.3	-24.1	x								21.5	2.77	1	0.0
CRC_Monitoring_152	24-Apr-13	41	14.8	-22.6		8-May-13	55	14	34		0	X	40	2.77	1	0.0
CRC_Monitoring_152	22-May-13	69	40.2	2.9		18-May-13	65	-4	45	y	32.4		41.7	2.77	1	0.0
CRC_Monitoring_152	20-Jun-13	98	58.8	21.4									119.9	2.77	1	0.0
CRC_Monitoring_153	21-Mar-13	0	80.6	0.0		12-Mar-13	-9	-9	57	n			84.1	5.43	2	74.2
CRC_Monitoring_153	3-May-13	43	71.2	-9.3		3-May-13	43	0	40		-29.8		176.7	5.43	2	74.2
CRC_Monitoring_153	4-Jun-13	75	57.1	-23.5	x	4-Jun-13	75	0	40		-29.8	X	246.9	5.43	2	74.2
CRC_Monitoring_153	27-Jun-13	98	88.9	8.3		27-Jun-13	98	0	43		-24.6		449	5.43	2	74.2
CRC_Monitoring_153	4-Sep-13	167	87.0	6.4		4-Sep-13	167	0	69		21.1		1073	5.43	2	74.2
CRC_Monitoring_154	25-Jan-13	0	16.4	0.0		29-Jan-13	4	4	105	n			496.9	1.50	0	17.0
CRC_Monitoring_154	25-Feb-13	31	42.3	25.9	x	15-Mar-13	49	18	121	y	15.2	X	666.8	1.50	0	17.0
CRC_Monitoring_154	26-Mar-13	60	57.7	41.3									789.3	1.50	0	17.0
CRC_Monitoring_155	3-Apr-13	0	35.6	0.0		8-Apr-13	5	5	107	n			634.7	2.47	0	21.1
CRC_Monitoring_155	11-Apr-13	8	63.2	27.7	x									2.47	0	21.1
CRC_Monitoring_155	9-May-13	36	65.3	29.7									1629	2.47	0	21.1
CRC_Monitoring_156	10-Apr-13	0	0.8	0.0		12-Apr-13	2	2	29	n			68.4	1.43	0	0.0
CRC_Monitoring_156	30-Apr-13	20	1.8	1.0	x								80.6	1.43	0	0.0
CRC_Monitoring_156	28-May-13	48	16.7	15.9									104.7	1.43	0	0.0
CRC_Monitoring_156	2-Jul-13	83	38.7	37.9		12-Jun-13	63	-20	36		24.1	X	111.5	1.43	0	0.0
CRC_Monitoring_158	28-Feb-14	0	14.6	0.0		10-Feb-14	-18	-18	29	n			6.8	1.63	1	57.8
CRC_Monitoring_158	27-Mar-14	27	0.0	-14.6	x	8-Apr-14	39	12	26		-10.3	X	3.11	1.63	1	57.8
CRC_Monitoring_158	7-May-14	68	5.2	-9.4									2.85	1.63	1	57.8
CRC_Monitoring_158	21-May-14	82	0.8	-13.8		20-May-14	81	-1	32		10.3		11.2	1.63	1	57.8
CRC_Monitoring_158	25-Jun-14	117	0.0	-14.6									23	1.63	1	57.8
CRC_Monitoring_159	17-Dec-13	0	17.8	0.0		18-Dec-13	1	1	172	n			262.8	1.40	0	8.8
CRC_Monitoring_159	22-Jan-14	36	36.2	18.4	x	3-Feb-14	48	12	198	y	15.1	X	373	1.40	0	8.8
CRC_Monitoring_159	19-Feb-14	64	45.2	27.3									523.4	1.40	0	8.8
CRC_Monitoring_160	18-Feb-14	0	47.4	0.0		5-Feb-14	-13	-13	107	n			280	10.63	1	0.0
CRC_Monitoring_160	20-Mar-14	30	46.7	-0.7	x								370.9	10.63	1	0.0
CRC_Monitoring_160	17-Apr-14	58	55.6	8.2		4-Apr-14	45	-13	126		17.8	X	659.4	10.63	1	0.0
CRC_Monitoring_161	4-Mar-14	0	4.5	0.0		26-Feb-14	-6	-6	29	n			22	2.83	1	0.6
CRC_Monitoring_161	9-Apr-14	36	16.1	11.6		23-Apr-14	50	14	24		-17.2	X	37.4	2.83	1	0.6
CRC_Monitoring_161	7-May-14	64	13.4	8.9	x	30-May-14	87	23	31	y	6.9		47.6	2.83	1	0.6
CRC_Monitoring_162	30-May-13	0	10.0	0.0		2-May-13	-28	-28	79	n			7.6	1.40	0	27.8
CRC_Monitoring_162	28-Jun-13	29	28.1	18.1	x								18.8	1.40	0	27.8
CRC_Monitoring_162	26-Jul-13	57	28.0	18.0		12-Jul-13	43	-14	108		36.7	X	24.1	1.40	0	27.8
CRC_Monitoring_163	14-Feb-13	0	1.4	0.0		23-Jan-13	-22	-22	190	n			1.4	4.00	1	1.8
CRC_Monitoring_163	15-Mar-13	29	0.0	-1.4	x	2-Apr-13	47	18	194		2.1	X	1.2	4.00	1	1.8
CRC_Monitoring_164	18-Feb-13	0	68.3	0.0		20-Feb-13	2	2	142	n			517.2	2.80	1	69.7
CRC_Monitoring_164	26-Mar-13	36	0.2	-68.1	x	28-Mar-13	38	2	140		-1.4	X	972.2	2.80	1	69.7
CRC_Monitoring_164	22-Apr-13	63	55.6	-12.7									1104	2.80	1	69.7
CRC_Monitoring_164	22-May-13	93	76.3	8.0		17-May-13	88	-5	152	y	7		1412	2.80	1	69.7
CRC_Monitoring_165	14-May-14	0	74.0	0.0		14-May-14	0	0	147				274.2	1.30	0	25.3
CRC_Monitoring_165	11-Jun-14	28	56.9	-17.1	x	23-Jun-14	40	12	176		19.7	X	830.2	1.30	0	25.3
CRC_Monitoring_165	9-Jul-14	56	72.5	-1.5						n			2207	1.30	0	25.3
CRC_Monitoring_166	14-May-13	0	22.6	0.0		23-May-13	9	9	48				57.5	1.57	0	16.6
CRC_Monitoring_166	24-May-13	10	54.5	31.9	x	10-Jul-13	57	47	79		64.6	X	63.7	1.57	0	16.6
CRC_Monitoring_168	3-Mar-14	0	83.5	0.0		5-Feb-14	-26	-26	85				421.4	1.40	0	81.0
CRC_Monitoring_168	30-Apr-14	58	92.5	9.0	x	17-Apr-14	45	-13	117	y	37.6	X	1065	1.40	0	81.0
CRC_Monitoring_169	2-Oct-13	0	48.3	0.0		4-Oct-13	2	2	108	n			342.8	1.40	0	19.8
CRC_Monitoring_169	9-Oct-13	7	48.5	0.1	x									1.40	0	19.8
CRC_Monitoring_169	6-Nov-13	35	65.6	17.2									794.8	1.40	0	19.8
CRC_Monitoring_169	5-Dec-13	64	74.3	25.9		21-Nov-13	50	-14	135	y	25	X	1144	1.40	0	19.8

Supplementary File 3

Experimental design	
Definition of experimental and control groups:	<p>Marker Discovery (From microarrays on tissue, new data and publically available dataset):</p> <p>Identification of markers:</p> <p>Cell bank (GSE86078; N=149)</p> <p>Normal Mucosa (GSE32146; N=20)</p> <p>Blood controls (GSE41169; N=34)</p> <p>In-silico Validation:</p> <p>Test set (GSE72752; N=63)</p> <p>Validation set (TCGA COREAD; N=446 / GSE48684; N=41)</p> <p>Tissue validation:</p> <p>Normal / matched cohort (N=32)</p> <p>Plasma matched cohort (N=51)</p> <p>cfDNA assessment:</p> <p>Prevalence assessment</p> <p>§ Aging cohort, self-declared healthy (N=50)</p> <p>§ mCRC cohort (N=135)</p> <p>§ Longitudinal cohort – baselines (N=47)</p> <p>Longitudinal assessment:</p> <p>§ Conventional chemotherapies (N=12)</p> <p>§ Targeted therapy (N=6)</p> <p>§ Clinical trial with temozolomide (N=29)</p> <p>All Cohorts and numbers are also reported in Figure 1.</p>
Assay carried out by core lab or investigator's lab?	All experiments were carried out in the investigator's laboratory. All DNA extraction and subsequent molecular characterization were carried out at the Department of Oncology of the Univesity of Turin."
Sample Description	
Samples used	Description and annotation of samples can be found in supplementary data 1A for the microarray analyses and in supplementary data 1E for the tissue and cfDNA samples.
Volume or mass of sample processed.	<p>Concerning the FFPE samples:</p> <p>§ the normal / tumor matched samples were macrodissected and a piece of FFPE block (~9mm³) was sent to the laboratory for extraction.</p> <p>§ the plasma matched tissues were remaining DNA samples extracted from two 10um slides (without tumour cellularity control) extracted using the QIA FFPE tissue kit (Qiagen).</p> <p>Concerning the cfDNA: One or two 6ml vacutainer tubes were collected from each timepoint of the CRC cohorts. Blood were processed within six hours as previously described (two steps centrifugation), and aliquoted into 1ml cryovials which were stored at -80°C until DNA extraction. These samples being used for collaborative project one milliliter of plasma was available for the current study.</p>
Microdissection or macrodissection.	<p>the normal / tumor matched samples were macrodissected.</p> <p>While FFPE tissue DNA (plasma matched tumours) were obtained through a previous project in which only the tumor extension was controlled without further dissection.</p>

Processing procedure	
If frozen—how and how quickly?	Plasma samples from CRC patients were frozen at -80°C within six hours after collection.
If fixed—with what, how quickly?	Fixation was performed in Paraformaldehyde 4%. Since tissue samples were retrieved from multiple collection center across Italie, it was not possible to retrieve the exact fixation duration period
Sample storage conditions and duration	<ul style="list-style-type: none"> - FFPE block were from archival tissue bank and have been stored between at room temperature in darkness up to 13 years prior processing (average=6 years). Cut of the block was performed prior to DNA extraction. - Plasma samples were kept at -80°C prior to extraction up to 8 years (average = 4 years).
Nucleic acid extraction	
Quantification—instrument/method.	<ul style="list-style-type: none"> - FFPE tissue DNA were quantified using Nanodrop system. - Circulating DNA were quantified by genome equivalent evaluated by qPCR using the LINE1 assay previously described in Siravegna et al. Nat Med. 2015.
Storage conditions: temperature, concentration, duration, buffer.	<ul style="list-style-type: none"> - All native / concentrated DNA were eluted in water and stored at +4°C.
Template structural information.	DNA were from FFPE tissue origin or from cell free circulating DNA. DNA were stored without further modifications prior to their bisulfite conversion for methylation analyses.
Template modification (digestion, sonication, preamplification, etc.).	Template were bisulfite converted as mentioned in material and methods using the EZ methylation Gold kit from Zymo Research according to manufacturers' procedure except for the elution step which was carried out in twice 20ul for cfDNA and in twice 40ul for tissue DNA.
Manufacturer of reagents used and catalogue number	<ul style="list-style-type: none"> - Qiagen - QIAamp DNA FFPE Tissue Kit - Cat No./ID: 56404 - Qiagen - QIAamp Circulating Nucleic Acid Kit - Cat No./ID: 55114 - Promega - Maxwell® RSC ccfDNA Plasma Kit - Cat No./ID:AS1480 - Zymo Research - EZ DNA Methylation-Gold™ Kit - Cat No./ID: D5006
Reaction volume	<ul style="list-style-type: none"> - Bisulfite conversion was carried out as follow: §FFPE DNA : input : 250ng output: 2x40ul §cfDNA : input: 20ul output: 2x20ul - 1st PCR amplification: FFPE and cfDNA : input : 2ul of bisulfite converted DNA per reaction final volume: 20ul - 2nd amplification (EmPCR): FFPE : Input : 3.5ul of 1st amplification (diluted at 1/20000) final volume: 20ul (+70ul oil) cfDNA : Input : 3.5ul of 1st amplification (diluted at 1/6000) final volume: 20ul (+70ul oil)

dPCR target information	
Amplicon length	C9orf50: 114bp EYA4: 96bp GRIA4: 106bp ITGA4: 76bp MAP3K14-AS1: 99bp MSC: 122bp SEPT9: 75bp
In silico specificity screen (BLAST, etc.)	<p>- EYA4: PCR product(s) on the bisulfite transformed sense chain Forward primer: GTGGATAGGATGGAAGTTT Reverse primer: CCCCCCACCTCCCTAC</p> <p>No PCR product should be generated.</p> <p>PCR product(s) on the bisulfite transformed antisense chain Forward primer: GTGGATAGGATGGAAGTTT Reverse primer: CCCCCCACCTCCCTAC</p> <p>1. Chromosome 6 (len: 96) 133241282 CCCAC CCCCACCTCCC TACACAAATA CAAAACTACC AACAAACACAA CACACACTCC AACCATTCCC AACTTCCACA CAAAACTTCC ATCCTATCCA C 133241378</p> <p>- GRIA4: PCR product(s) on the bisulfite transformed sense chain Forward primer: GGGTTGGTGTAGGTTTGGT Reverse primer: CTCCCCCTTACTTTCTCACATACACACAA</p> <p>1. Chromosome 11 (len: 106) 105610527 GG GTTGGTGTAG GTTTGGTGGG GGATGTTGGT TGATGTGAGT TGGAGAGTGT GTGTGGTGGT TGTGGTGTTA GTGT TGTGTGT GTATGTGAGA AAGTAAGGGG TGAG 105610633</p> <p>PCR product(s) on the bisulfite transformed antisense chain Forward primer: GGGTTGGTGTAGGTTTGGT Reverse primer: CTCCCCCTTACTTTCTCACATACACACAA</p> <p>No PCR product should be generated.</p> <p>- ITGA4: PCR product(s) on the bisulfite transformed sense chain Forward primer: GGGGAAGAAAGTTTAAAGAGATGAG Reverse primer: TCCTCTCCCTCTCTCCT</p> <p>No PCR product should be generated.</p> <p>PCR product(s) on the bisulfite transformed antisense chain Forward primer: GGGGAAGAAAGTTTAAAGAGATGAG Reverse primer: TCCTCTCCCTCTCTCCT</p> <p>1. Chromosome 2 (len: 76) 181457503 T CCTCTCCCT CTCTCCTTCC TTTAACCCAC TAACACCAAA CACACTACAC CTCATCTCTT AAAACAATTCT TCCCC 181457579</p> <p>- MAP3K14-AS1: PCR product(s) on the bisulfite transformed sense chain Forward primer: GGATTGGTGGGAGTTGGAATATTAGA Reverse primer: ACCTAAATACCACTCCCTACCTATAAAAT</p> <p>1. Chromosome 17 (len: 103) 45262097 GGATTG GTGGG GTTG GAAATATTAG AGGTGTTGGT GTTGTGATG TTGTTGTTTT TGTGATGTTT TTGTTGTGAT TTTATAGGTA GGGAGTTGTA TTTAGGT 45262200</p> <p>PCR product(s) on the bisulfite transformed antisense chain Forward primer: GGATTGGTGGGAGTTGGAATATTAGA Reverse primer: ACCTAAATACCACTCCCTACCTATAAAAT</p> <p>No PCR product should be generated.</p>

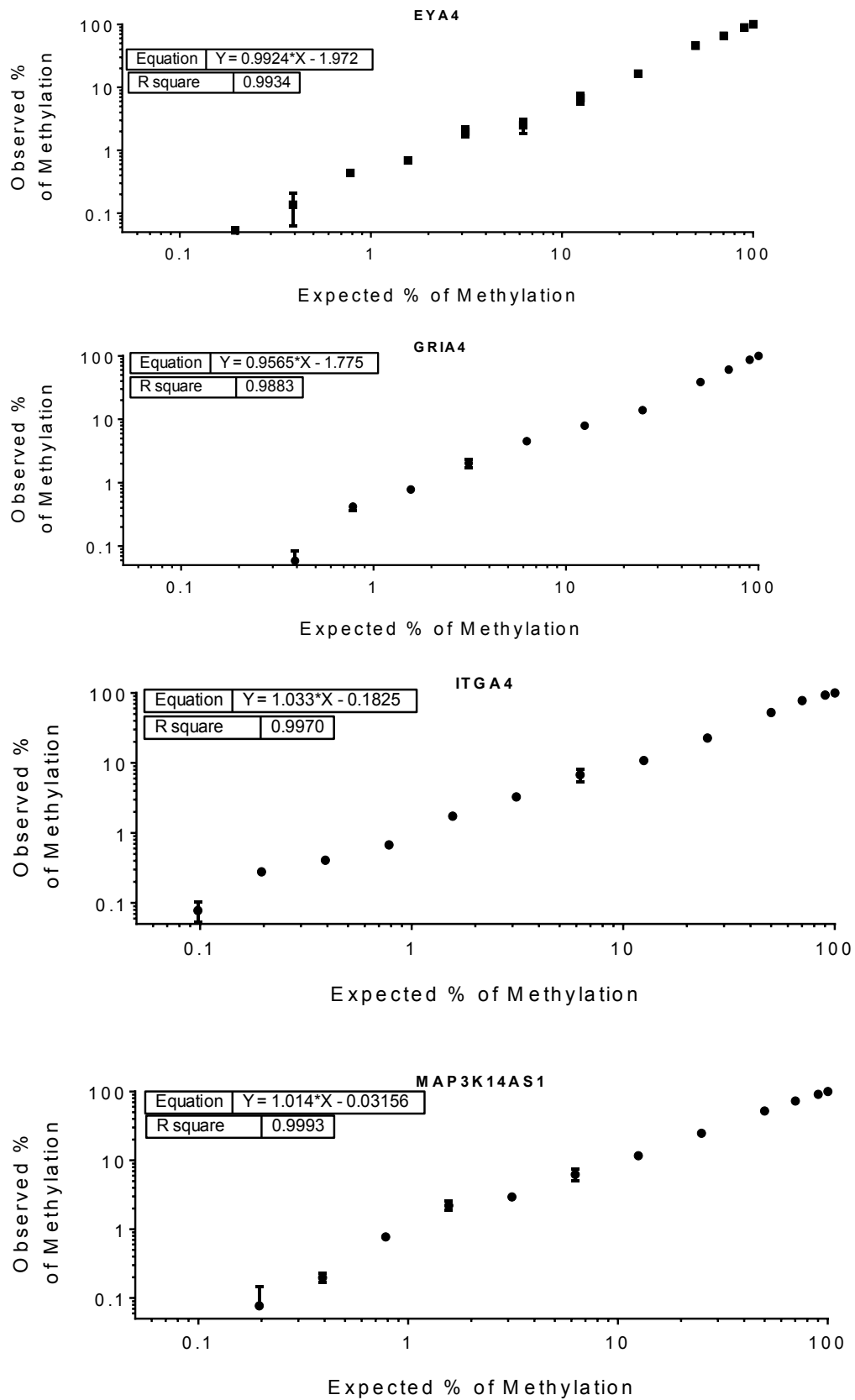
	<p>- MSC:</p> <p>PCR product(s) on the bisulfite transformed sense chain Forward primer: AGGGTTTAGTAGTAGAGTGTAAAGT Reverse primer: AAACACCCAAAACAACTAATCT</p> <p>No PCR product should be generated.</p> <p>PCR product(s) on the bisulfite transformed antisense chain Forward primer: AGGGTTTAGTAGTAGAGTGTAAAGT Reverse primer: AAACACCCAAAACAACTAATCT</p> <p>1. Chromosome 8 (len: 122) 71843764 AAA CACCCAAAAC AAACTAATCT TAAACCTAAA AAAAAGTTTA CTCAACACAC ACATCCAAAC ACACTCACAA ACATTAACCA CATTCCACTA CAACTACTTA CACTCTACAA CTAAACCCT</p> <p>71843886</p>
Pseudogenes, or other homologs	Not found.
Secondary structure analysis of amplicon and GC content.	<p>- EYA4 amplicon:</p> <p>SEQUENCE 5'- CCC ACC TCC CTA CAC AAA TAC AAA ACT ACC AAC AAC ACA ACA CAC ACT CCA ACC ATT CCC AAC TTC CAC ACA AAA CTT CCA TCC TAT CCA C -3' COMPLEMENT 5'- GTG GAT AGG ATG GAA GTT TTG TGT GGA AGT TGG GAA TGG TTG GAG TGT GTG TTG TGT TGT TGG TAG TTT TGT ATT TGT GTA GGG AGG TGG G -3'</p> <p>LENGTH 91 GC CONTENT 45.1 % MELT TEMP 71 °C MOLECULAR WEIGHT 27328.8 g/mole EXTINCTION COEFFICIENT 856400 L/(mole·cm) nmole/OD260: 1.17 µg/OD260: 31.91</p> <p>- GRIA4 amplicon:</p> <p>SEQUENCE 5'- GGG TTG GTG TAG GTT TGT TGG GGG ATG TTG GTT GAT GTG AGT TGG AGA GTG TGT GTG GTT GTG GTG TTA GTG TTT GTG TGT ATG TGA GAA AGT AAG GGG TGA G -3' COMPLEMENT 5'- CTC ACC CCT TAC TTT CTC ACA TAC ACA CAA ACA CTA ACA CCA CAA CCA CCA CAC ACA CTC TCC AAC TCA CAT CAA CCA ACA TCC CCC AAC AAA CCT ACA CCA ACC C -3'</p> <p>LENGTH 106 GC CONTENT 48.1 % MELT TEMP 71.9 °C MOLECULAR WEIGHT 33593.6 g/mole EXTINCTION COEFFICIENT 1053700 L/(mole·cm) nmole/OD260: 0.95 µg/OD260: 31.88</p> <p>- ITGA4 amplicon:</p> <p>SEQUENCE 5'- TCC TCT TCC CTC TCT CCT TCC TTT AAC CCA CTA ACA CCA AAC ACA CTA CAC CTC ATC TCT TAA AAC ATT CTT CCC C -3' COMPLEMENT 5'- GGG GAA GAA TGT TTT AAG AGA TGA GGT GTA GTG TGT TTG GTG TTA GTG GGT TAA AGG AAG GAG AGA GGG AAG AGG A -3'</p> <p>LENGTH 76 GC CONTENT 44.7 % MELT TEMP 69.8 °C MOLECULAR WEIGHT 22717.8 g/mole EXTINCTION COEFFICIENT 669200 L/(mole·cm) nmole/OD260: 1.49 µg/OD260: 33.95</p>

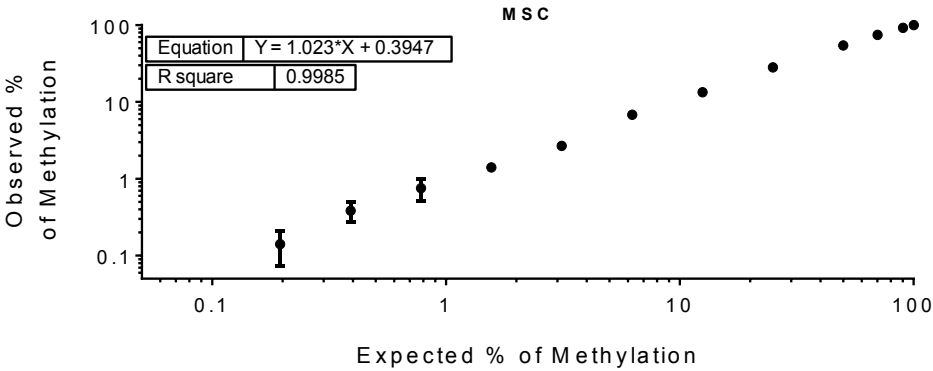
	<p>- MAP3K14-AS1 amplicon:</p> <p>SEQUENCE 5'- GGA TTG GTG GGT GTT GGA AAT ATT AGA GGT GTT GGT GTT TGT GAT GTT GTT GTT TTT GTG ATG TTT TTG TTG TGA TTT TAT AGG TAG GGA GTT GTA TTT AGG T -3'</p> <p>COMPLEMENT 5'- ACC TAA ATA CAA CTC CCT ACC TAT AAA ATC ACA ACA AAA ACA TCA CAA AAA CAA CAA CAT CAC AAA CAC CAA CAC CTC TAA TAT TTC CAA CAC CCA CCA ATC C -3'</p> <p>LENGTH 103</p> <p>GC CONTENT 35.9 %</p> <p>MELT TEMP 70 °C</p> <p>MOLECULAR WEIGHT 32339.9 g/mole</p> <p>EXTINCTION COEFFICIENT 1005700 L/(mole·cm)</p> <p>nmole/OD260: 0.99</p> <p>µg/OD260: 32.16</p> <p>- MSC amplicon:</p> <p>SEQUENCE 5'- AAA CAC CCA AAA CAA ACT AAT CTT AAA CCT AAA AAA AAC TTT ACT CAA CAC ACA CAT CCA AAC ACA CTC ACA AAC ATT AAC CAC ATT CCA CTA CAA CTA CTT ACA CTC TAC AAC TAA ACC CT -3'</p> <p>COMPLEMENT 5'- AGG GTT TAG TTG TAG AGT GTA AGT AGT TGT AGT GGA ATG TGG TTA ATG TTT GTG AGT GTG TTT GGA TGT GTG TGT TGA GTA AAG TTT TTT TTA GGT TTA AGA TTA GTT TGT TTT GGG TGT TT -3'</p> <p>LENGTH 122</p> <p>GC CONTENT 33.6 %</p> <p>MELT TEMP 70.6 °C</p> <p>MOLECULAR WEIGHT 36957.2 g/mole</p> <p>EXTINCTION COEFFICIENT 1201200 L/(mole·cm)</p> <p>nmole/OD260: 0.83</p> <p>µg/OD260: 30.77</p>
dPCR oligonucleotides	
Primer sequences	<p>- C9orf50:</p> <p>FW [TCCCGCGAAATTAATACGAC] ATTTAAGGAATTTGTTGGGGAGGA</p> <p>RV [GCTGGAGCTCTGCAGCTA] CCCAAACAACCAAAACCTAA</p> <p>- EYA4:</p> <p>FW [TCCCGCGAAATTAATACGAC] GTGGATAGGATGGAAGTTT</p> <p>RV [GCTGGAGCTCTGCAGCTA] CCCCCCACCCTCCCTAC</p> <p>- GRIA4:</p> <p>FW [TCCCGCGAAATTAATACGAC] GGGTTGGTGTAGGTTTGTT</p> <p>RV [GCTGGAGCTCTGCAGCTA] CTCCCCCTTACTTTCTCACATACACACAA</p> <p>- ITGA4</p> <p>FW [TCCCGCGAAATTAATACGAC] GGGGAAGAAAGTTTAAAGAGATGAG</p> <p>RV [GCTGGAGCTCTGCAGCTA] TCCTCTTCCCTCTCTCCT</p> <p>- MAP3K14-AS1:</p> <p>FW [TCCCGCGAAATTAATACGAC] GGATTGGTGGGAGTTGGAAATATTAGA</p> <p>RV [GCTGGAGCTCTGCAGCTA] ACCTAAATACCACTCCCTACCTATAAAAT</p> <p>- MSC:</p> <p>FW [TCCCGCGAAATTAATACGAC] AGGGTTTAGTAGTAGAGTGTAAGT</p> <p>RV [GCTGGAGCTCTGCAGCTA] AAACACCAAAACAACTAATCT</p> <p>- SEPT9:</p> <p>FW [TCCCGCGAAATTAATACGAC] GGATTTAGAAGGTGGGTGTTGG</p> <p>RV [GCTGGAGCTCTGCAGCTA] CCAAACCCACCCCAAAATCCTCTC</p>
Probe sequences	<p>- C9orf50</p> <p>Methylated-Probe /5A1ex647N/AACGCGAACGCCCCGAA</p> <p>Unmethylated-Probe /5A1ex488N/AACACAAACACCCCCAAA</p> <p>- EYA4</p> <p>Methylated-Probe /5A1ex647N/GACCGTTCCCGACTTCCGC</p> <p>Unmethylated-Probe /5A1ex488N/CACTCCAACCATTCCCA</p> <p>- GRIA4</p> <p>Methylated-Probe /5A1ex647N/AACGCCGCGACGCCACAC</p>

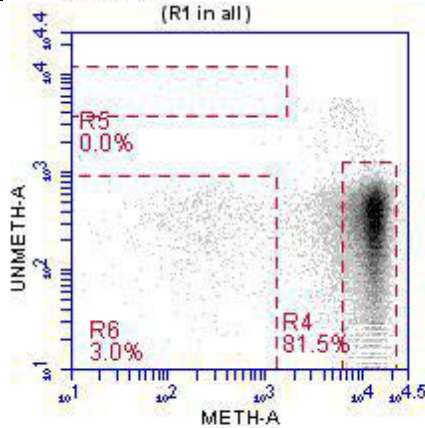
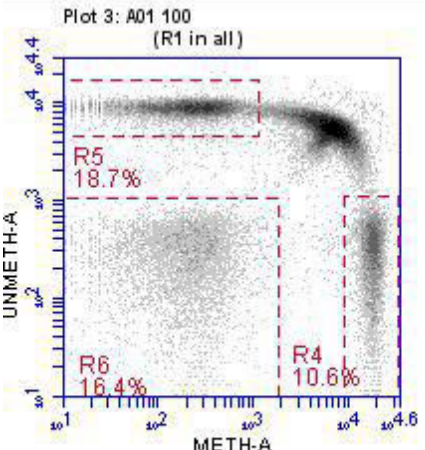
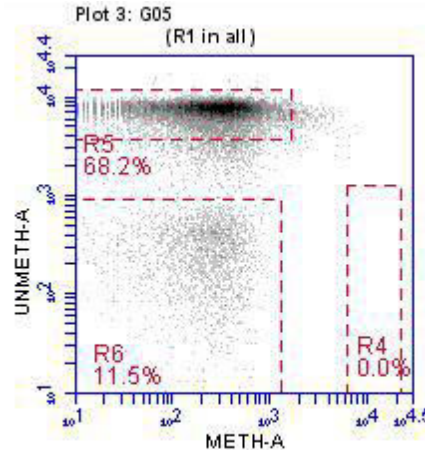
	Unmethylated-Probe /5Alex488N/CACCACAACCACCACACACA - <u>ITGA4</u> Methylated-Probe /5Alex647N/ACCGCTAACGCCGAACACGCTA Unmethylated-Probe /5Alex488N/ACCCACTAACACCAAACACACTA - <u>MAP3K14-AS1</u> Methylated-Probe /5Alex647N/CGACGACATCACAAACACCGACG Unmethylated-Probe /5Alex488N/CAACAACATCACAAACACCAACA - <u>MSC</u> Methylated-Probe /5Alex647N/CGCATCCGAACACGCTCAC Unmethylated-Probe /5Alex488N/ACACATCCAAACACACTCAC - <u>SEPT9</u> Methylated-Probe /5Alex647N/CCGCGACCGCAACAACC Unmethylated-Probe /5Alex488N/CCACAACCACAACAACC
Manufacturer of oligonucleotides	All primers and probes were ordered through IDT.
Purification method	Primers were ordered desalted while probes were purified with HPLC and modified with fluorescent dye at 5' (/5Alex647N/ for methylated probe ; /5Alex488N/ for unmethylated probes)
dPCR protocol	
Complete reaction conditions	<u>- Mg concentrations:</u> - C9orf50: 1.5mM - EYA4: 2 mM - GRIA4: 1.5 mM - ITGA4: 2.5 mM - MAP3K14-AS1: 2.5 mM - MSC: 1.5 mM - SEPT9: 1 mM <u>- Tm:</u> - C9orf50: 50°C - EYA4: 54°C - GRIA4: 62°C - ITGA4: 50°C - MAP3K14-AS1: 50°C - MSC: 58°C - SEPT9: 56°C
Plates/tubes Catalogue No and manufacturer	- 1 st amplification: 8-tube strips for qPCR with individually attached, optically clear, flat caps (VWR – Cat No: 211-0338) - EmPCR: PCR-PLATE 96-WELL NON-SKIRTED STP (VWR – Cat No: 732-2387)
Complete thermocycling parameters.	- <u>1st amplification:</u> 94°C 2 min ----- ① ----- 94°C 30 sec Tm 15 sec Tm:see above 72°C 15 sec -- go to ① -- x39 72°C 5 min - <u>EmPCR amplification:</u> 94.0 2 min ----- ① ----- 94.0 10 sec 68.0 50 sec 70.0 80 sec

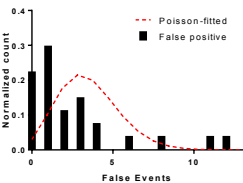
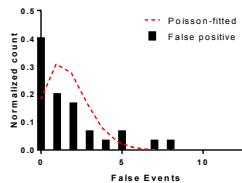

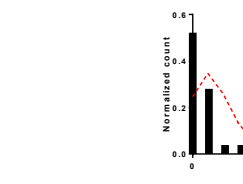
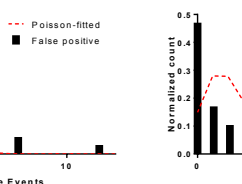
	<pre>-- go to ① -- x3 ----- ② ----- 94.0 10 sec 65.0 50 sec 70.0 80 sec -- go to ② -- x3 ----- ③ ----- 94.0 10 sec 62.0 50 sec 70.0 80 sec -- go to ③ -- x3 ----- ④ ----- 94.0 10 sec 59.0 50 sec 70.0 80 sec -- go to ④ -- x50 70.0 2 min</pre>
Reaction setup	<p>Buffer, Magnesium and Taq are from Platinum® Taq DNA Polymerase (ref. Thermo Fisher 10966018)</p> <p>dNTP are from dNTP Set (100 mM) (ref. Thermo Fisher 10297018)</p> <p>- <u>1st amplification:</u></p> <p>Buffer: 1X Mg: See above dNTP: 300nM primers: 200nM Taq: 0.75 unit Template : 2 ul (variable concentration) H2O: qsp 20 ul</p> <p>- <u>EmPCR (Ref: Diehl 2006, Nat methods):</u></p> <p>Buffer: 1X Mg: 5 mM dNTPs: 200uM Tag 2: 8 uM Tag1: 50 nM Beads: 6.107 Taq Platinum: 2 units DNA: ~25 pg H2O: qsp 20 ul</p>
Manufacturer of instrument	<p>Amplification performed on a Bio-rad C1000 thermocycler.</p> <p>Reading was performed on a BD – Accuri C6 flow cytometer.</p>

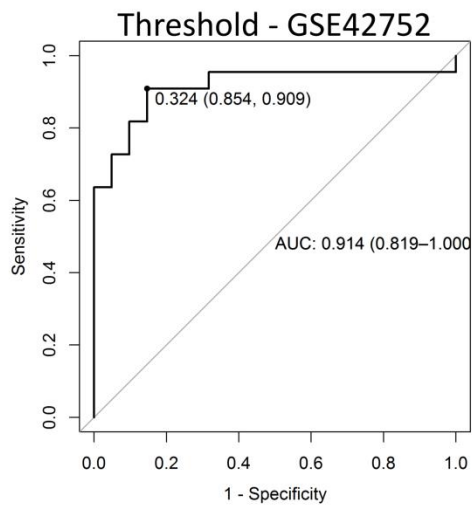
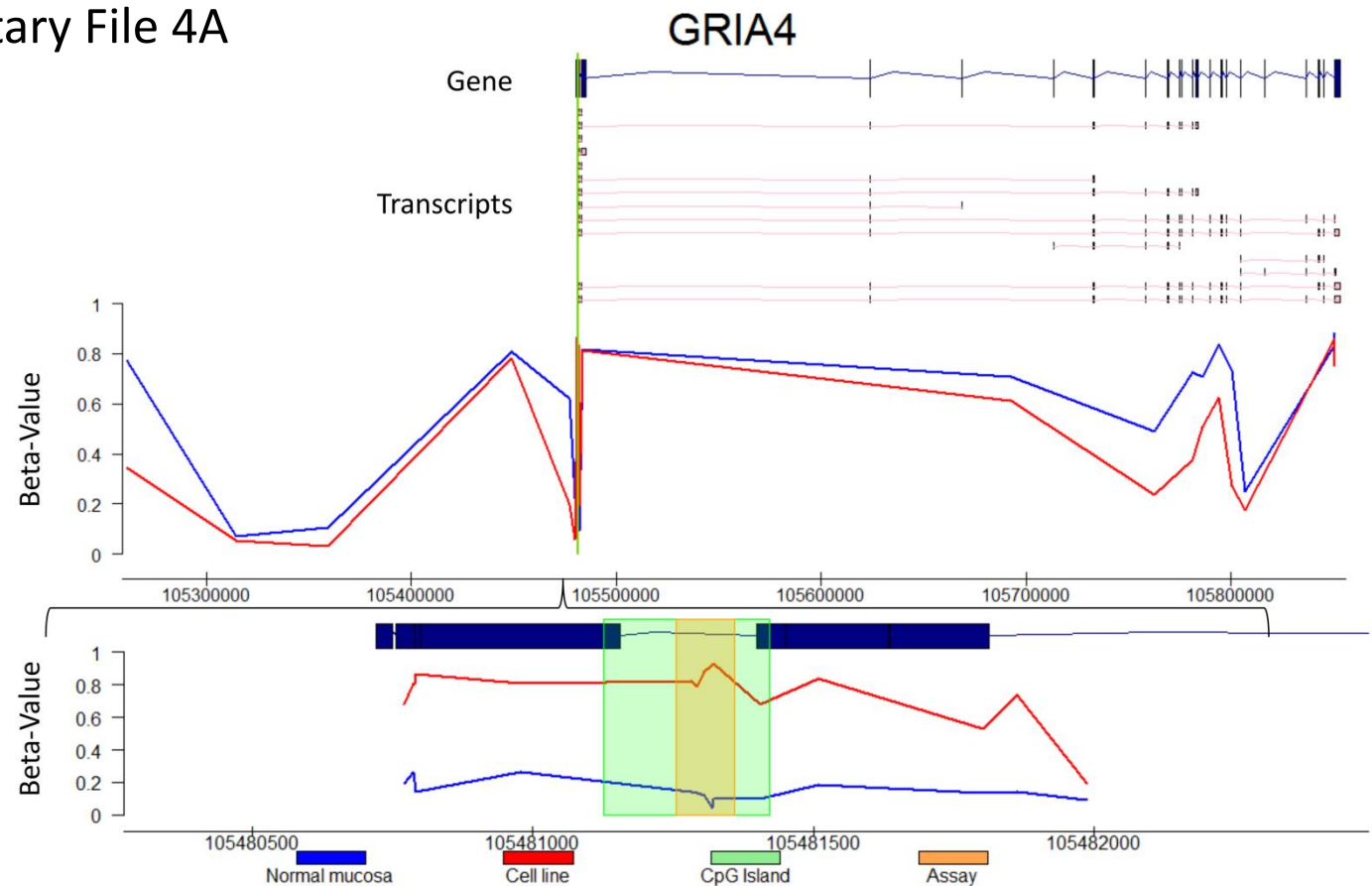
Optimization data
for the assay



	 <p>Equation $Y = 1.023 \cdot X + 0.3947$ R square 0.9985</p> <p>MSC</p> <p>Observed % of Methylation</p> <p>Expected % of Methylation</p>
Limit of detection of calibration control.	<p>The Limit of detection using calibration control (artificial DNA) was down to:</p> <ul style="list-style-type: none"> § EYA4: 0.18% § GRIA4: 0.18% § ITGA4: 0.09% § MAP3K14-AS1: 0.18% § MSC: 0.09%
Mean copies per partition	<p>The average number of events over all tissue samples assessed were:</p> <ul style="list-style-type: none"> - EYA4: 9767 events - GRIA4: 5995 events \ - ITGA4: 4603 events - MAP3K14-AS1: 9308 events - MSC: 7846 events <p>The average number of events over all cfDNA samples assessed were:</p> <ul style="list-style-type: none"> - EYA4: 18939 events - GRIA4: 11401 events \ - ITGA4: 3677 events - MAP3K14-AS1: 15963 events - MSC: 16584 events <p>In the prevalence dataset only comparing normal self-declared healthy samples (H) from cancer samples (C):</p> <ul style="list-style-type: none"> - EYA4: H= 8560 events – C= 20594 - GRIA4: H= 4464 events – C= 12562 - ITGA4: H= 2590 events – C= 3855 - MAP3K14-AS1: H= 4715 events – C= 17798 - MSC: H= 9216 events – C= 17777
dPCR analysis program (source, version)	BD Accuri C6 software Version 1.0.264.21
Outlier identification and disposition.	<p>Non amplified samples were amplified a 3rd time and in case of failure were annotated as NA.</p> <p>Replicates with more than 20% variation in cfDNA were re-amplified.</p>
Results of no-template controls	No-template control had to show less than 100 cumulated events (Methylated and Unmethylated), or the run was reperformed.
Examples of positive(s) and negative experimental results	<p>For each graph: R5 Unmethylated events; R4 Methylated Events; R6 Empty Beads.</p> <ul style="list-style-type: none"> - Fully methylated sample:

	 <p>- Intermediate methylation: (37.1%)</p>  <p>- Fully unmethylated:</p> 
normalization method	<p>Percentage of methylation was calculated on the number of events obtained in the methylated gate (R4) / number of cumulated events (events in methylated (R4) and unmethylated gate (R5)).</p> $\text{Methylation value} = \frac{R4}{(R4 + R5)} \times 100$
biological replicates	Not applicable.
Number and stage of technical replicates.	<p>Replication was performed at the 1st amplification stage. <i>i.e.</i> 1 plasma / tissue sample → 1 DNA → 1 BisDNA → 2 1st PCR /assay → 2 EmPCR /assay → 2 well in FACs.</p>
Repeatability	The repeatability was assessed for tissue and cfDNA separately, considering the

(intraassay variation) & Experimental variance	<p>average standard deviation [± SD] on all assessed samples:</p> <ul style="list-style-type: none">- In tissue DNA:<ul style="list-style-type: none">§ EYA4: 4.36 % [± 2.84]§ GRIA4: 3.72 % [± 3.08]§ ITGA4: 2.97 % [± 2.47]§ MAP3K14-AS1: 3.24 % [± 3.14]§ MSC: 5.61 [± 4.67]- In cfDNA:<ul style="list-style-type: none">§ EYA4: 3.29 % [± 4.24]§ GRIA4: 4.07 % [± 4.78]§ ITGA4: 3.72 % [± 4.70]§ MAP3K14-AS1: 3.05 % [± 4.19]§ MSC: 2.90 % [± 4.26]																														
Limit of blank	<div><div><div><p>EYA4</p></div><div><p>GRIA4</p></div><div><p>ITGA4</p></div><div><p>MAPK314-AS1</p></div><div><p>MSC</p></div></div><p>The limit of blank was calculated using the negative template samples which displayed some output and on five ultramer samples corresponding to the fully unmethylated template. Since the distribution of the false events does not always follow a Poisson distribution, an empirical estimate for the 95% upper confidence limit of the data was estimated (as applied in Taly <i>et al.</i> Clin Chem 2013):</p><table><tr><th></th><th>N</th><th>λ</th><th>LOB (Poisson)</th><th>LOB (empirical)</th></tr><tr><td>EYA4</td><td>28</td><td>3.6</td><td>5</td><td>6</td></tr><tr><td>GRIA4</td><td>30</td><td>1.7</td><td>3</td><td>3</td></tr><tr><td>ITGA4</td><td>27</td><td>1.5</td><td>2</td><td>3</td></tr><tr><td>MAP3K14-AS1</td><td>29</td><td>1.9</td><td>3</td><td>3</td></tr><tr><td>MSC</td><td>30</td><td>2.4</td><td>3</td><td>4</td></tr></table></div>		N	λ	LOB (Poisson)	LOB (empirical)	EYA4	28	3.6	5	6	GRIA4	30	1.7	3	3	ITGA4	27	1.5	2	3	MAP3K14-AS1	29	1.9	3	3	MSC	30	2.4	3	4
	N	λ	LOB (Poisson)	LOB (empirical)																											
EYA4	28	3.6	5	6																											
GRIA4	30	1.7	3	3																											
ITGA4	27	1.5	2	3																											
MAP3K14-AS1	29	1.9	3	3																											
MSC	30	2.4	3	4																											

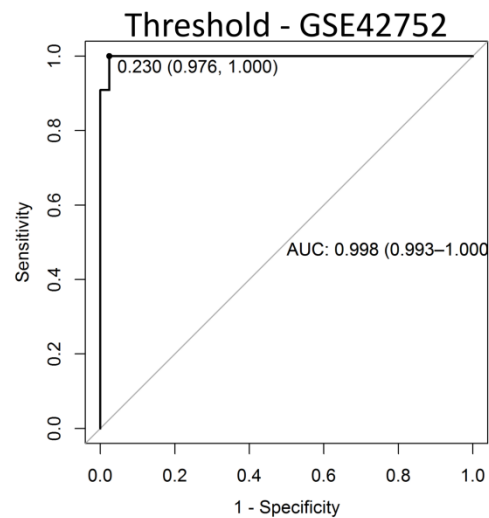
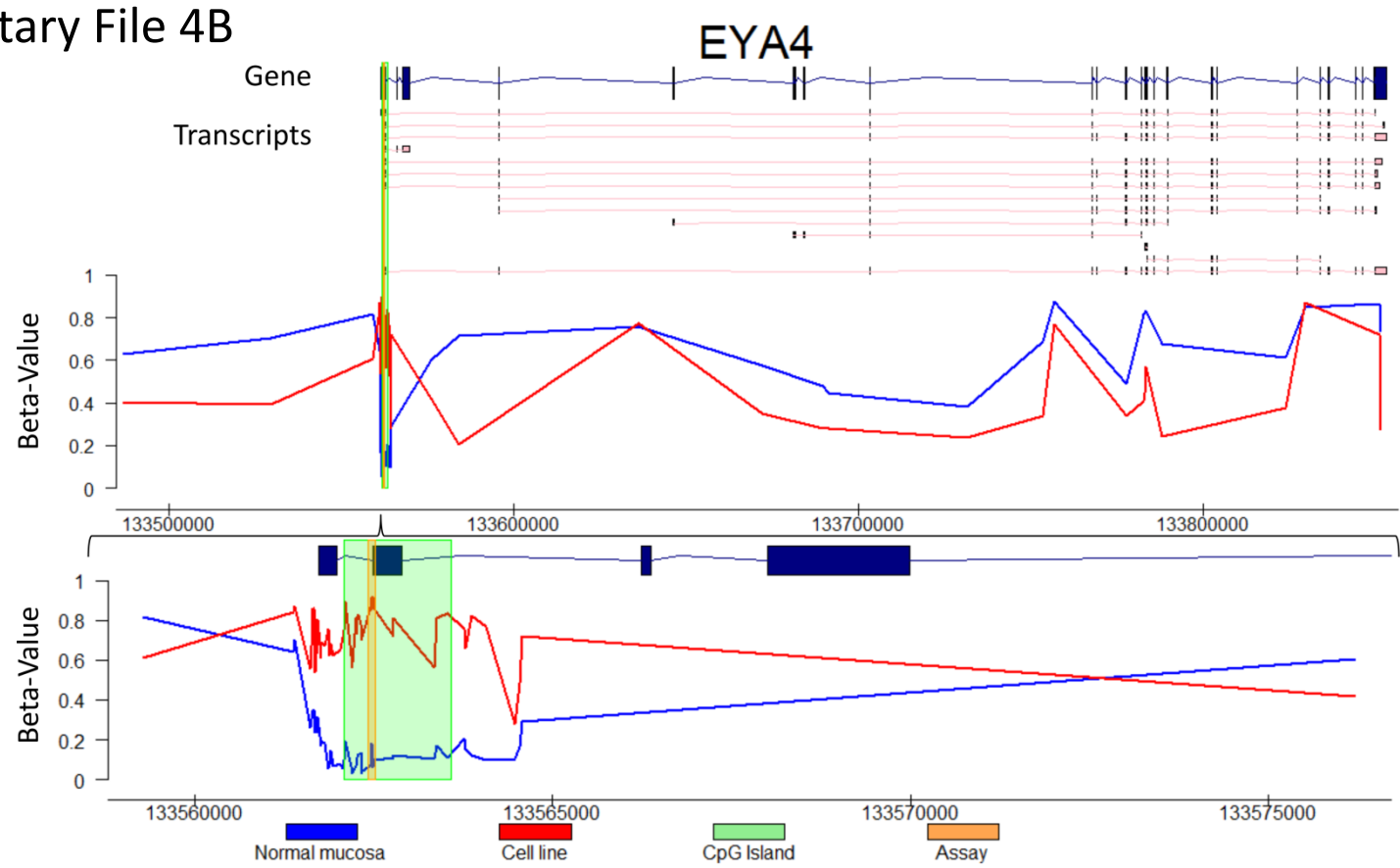


TCGA COREAD

p-value < 2e-16

	<0.324	>0.324	NPV	PPV
Normal	45	0	0.7	1
Cancer	16	399		

Supplementary File 4B



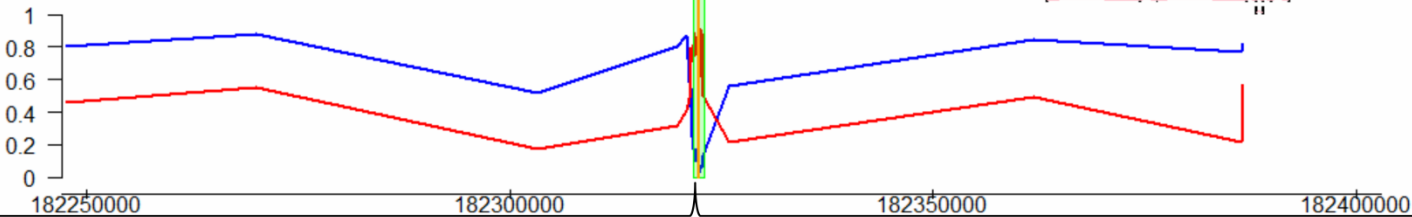
TCGA COREAD

p-value < 2e-16

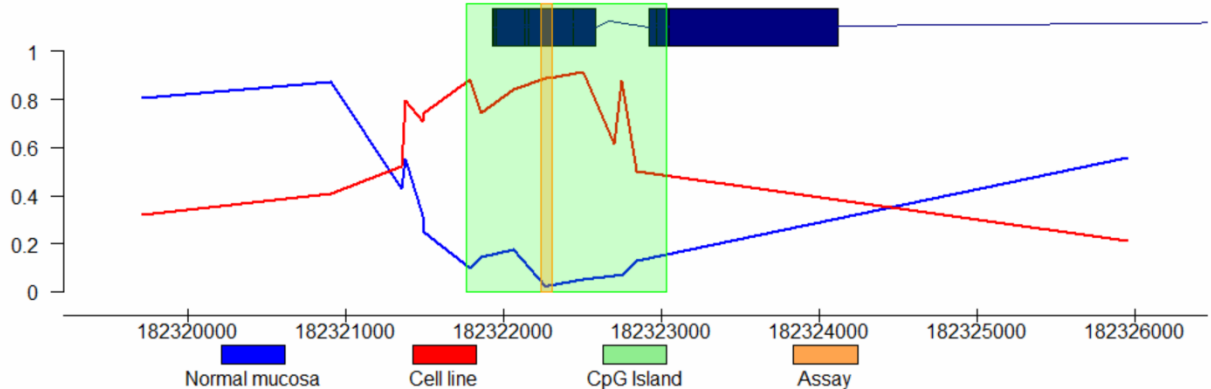
	<0.230	>0.230	NPV	PPV
Normal	45	0	0.6	1
Cancer	26	389		

ITGA4

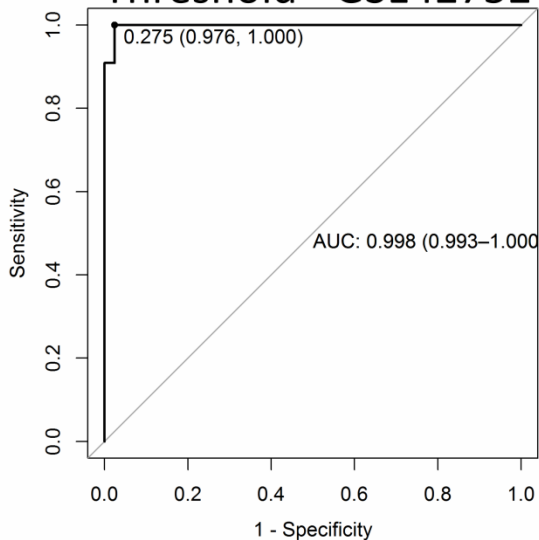
Beta-Value



Beta-Value

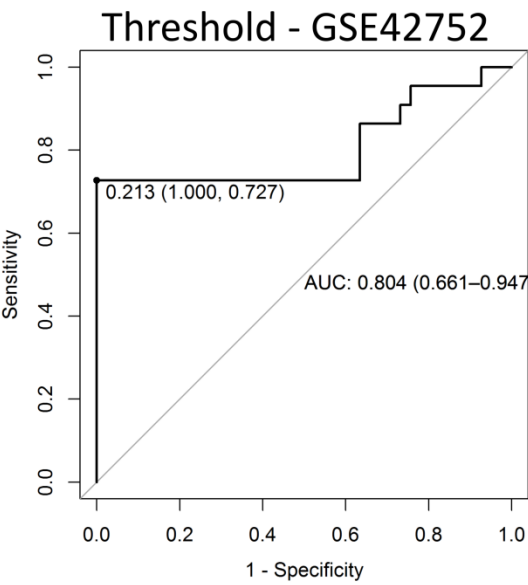
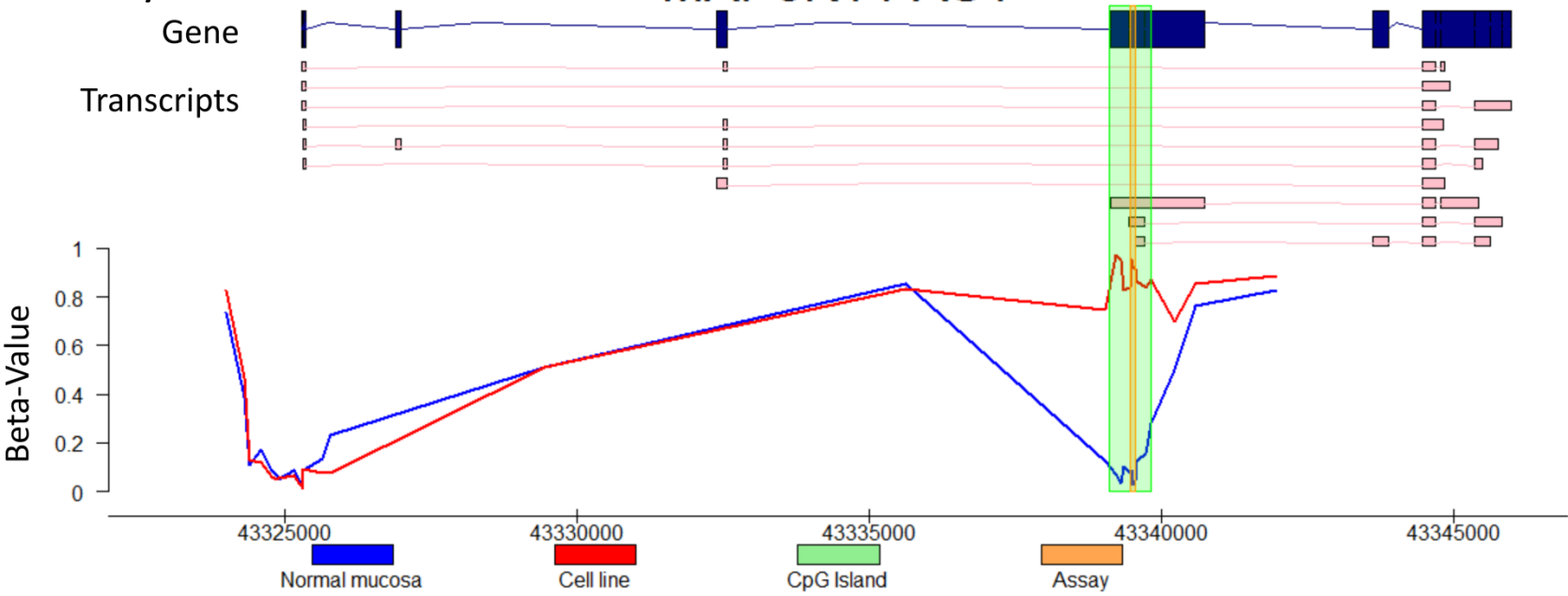


Threshold - GSE42752



TCGA COREAD

p-value < 2e-16	<0.275	>0.275	NPV	PPV
Normal	45	0	0.8	1
Cancer	9	403		

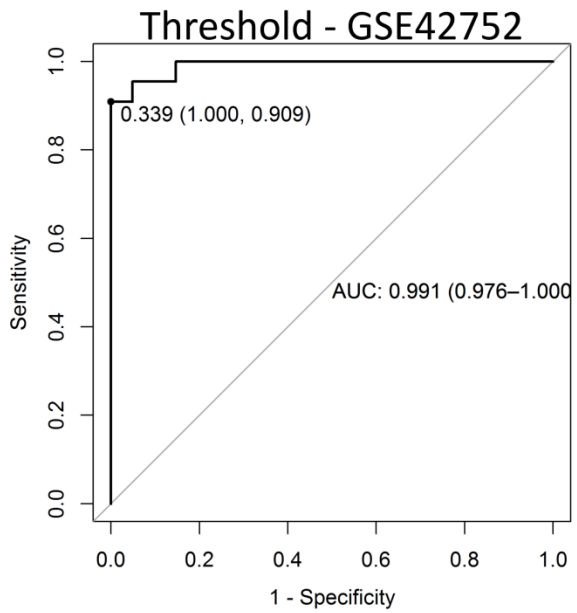
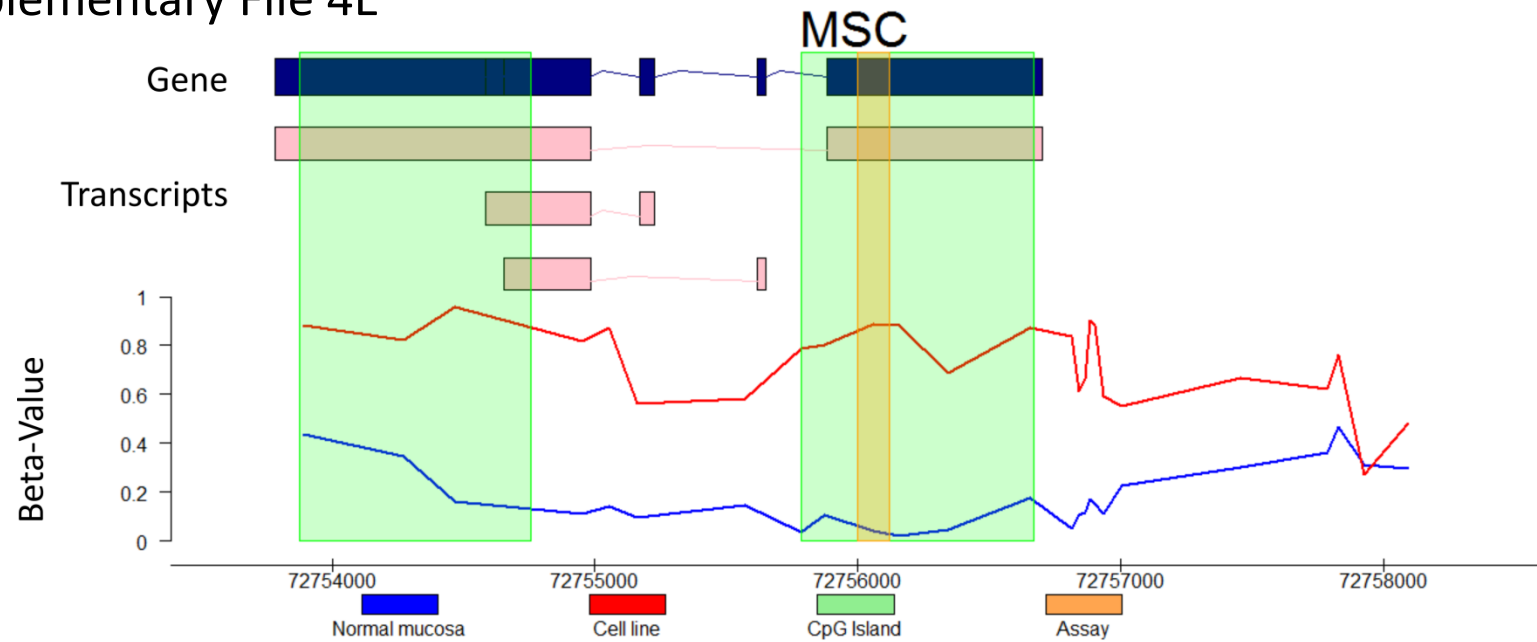


TCGA COREAD

p-value < 2e-16

	<0.213	>0.213	NPV	PPV
Normal	45	0	0.5	1
Cancer	39	376		

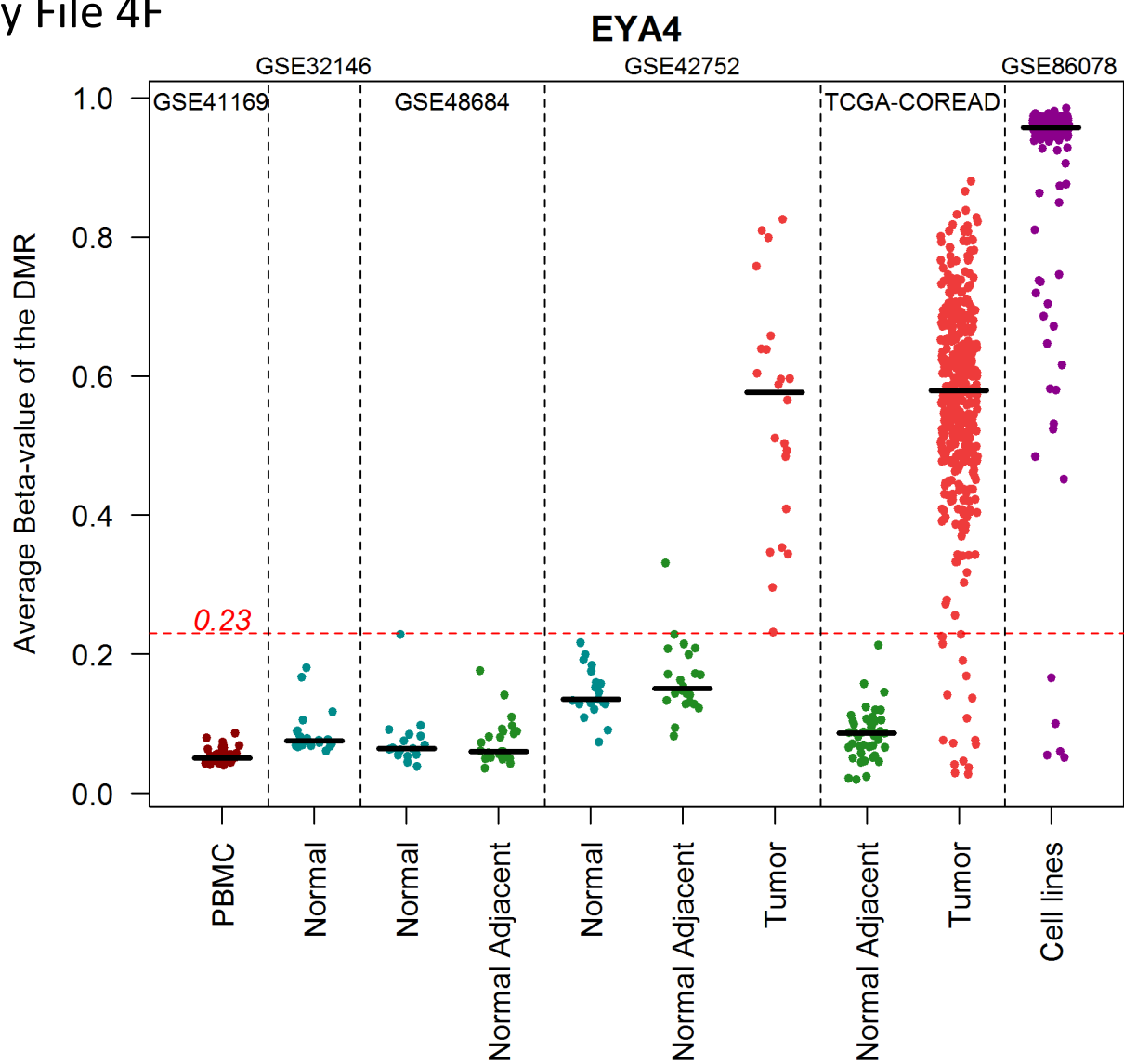
Supplementary File 4E

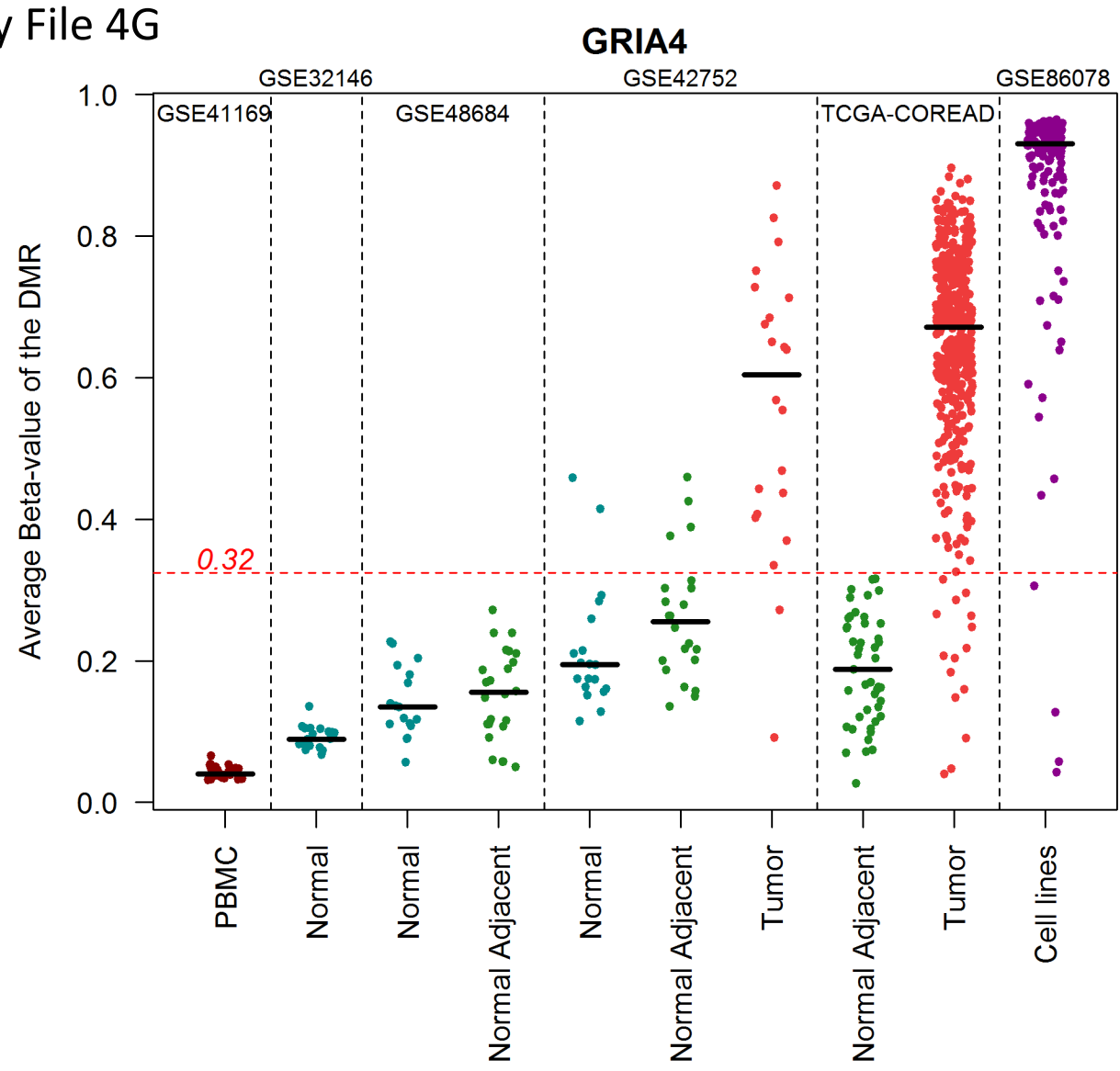


TCGA COREAD

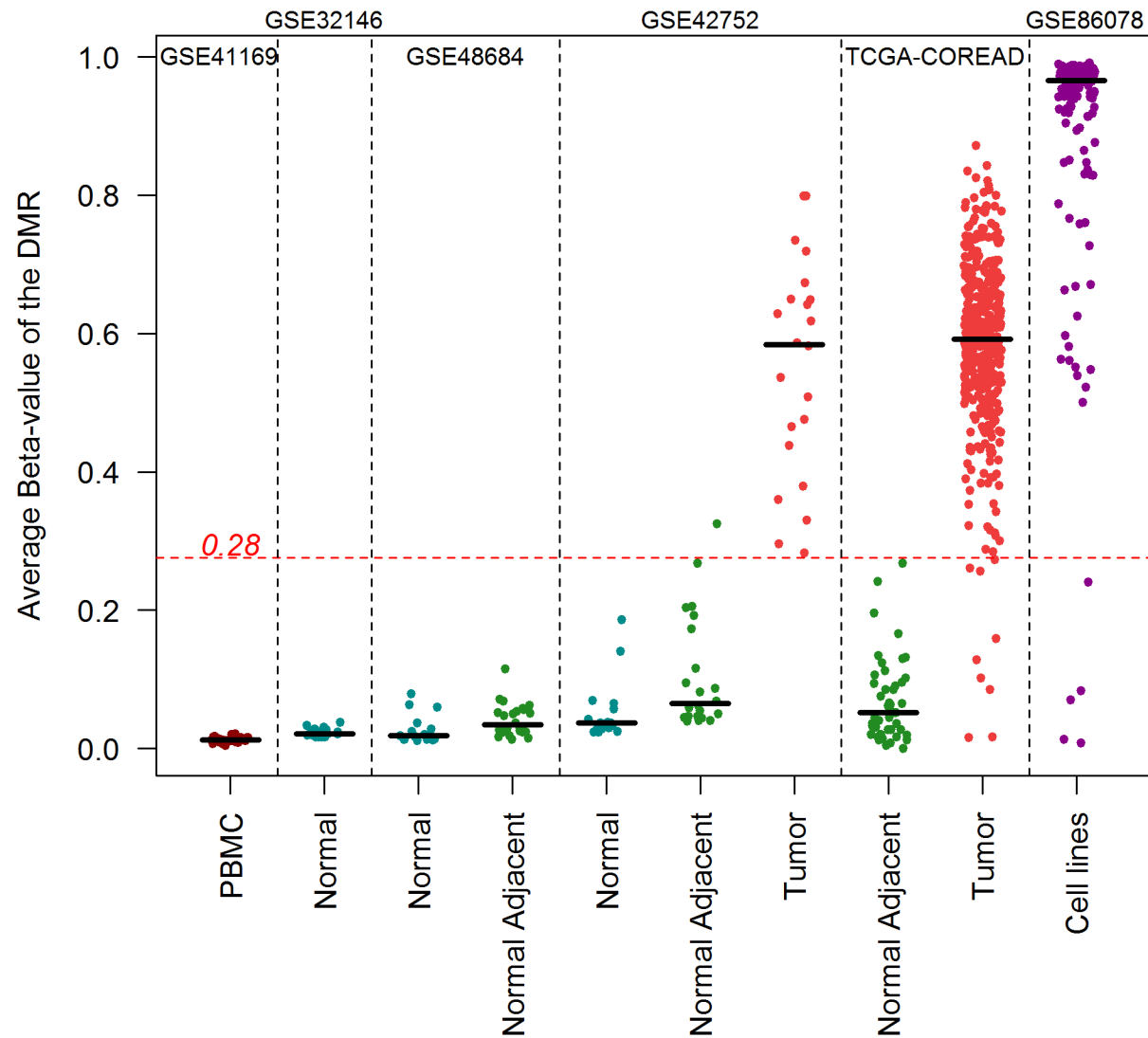
p-value < 2e-16

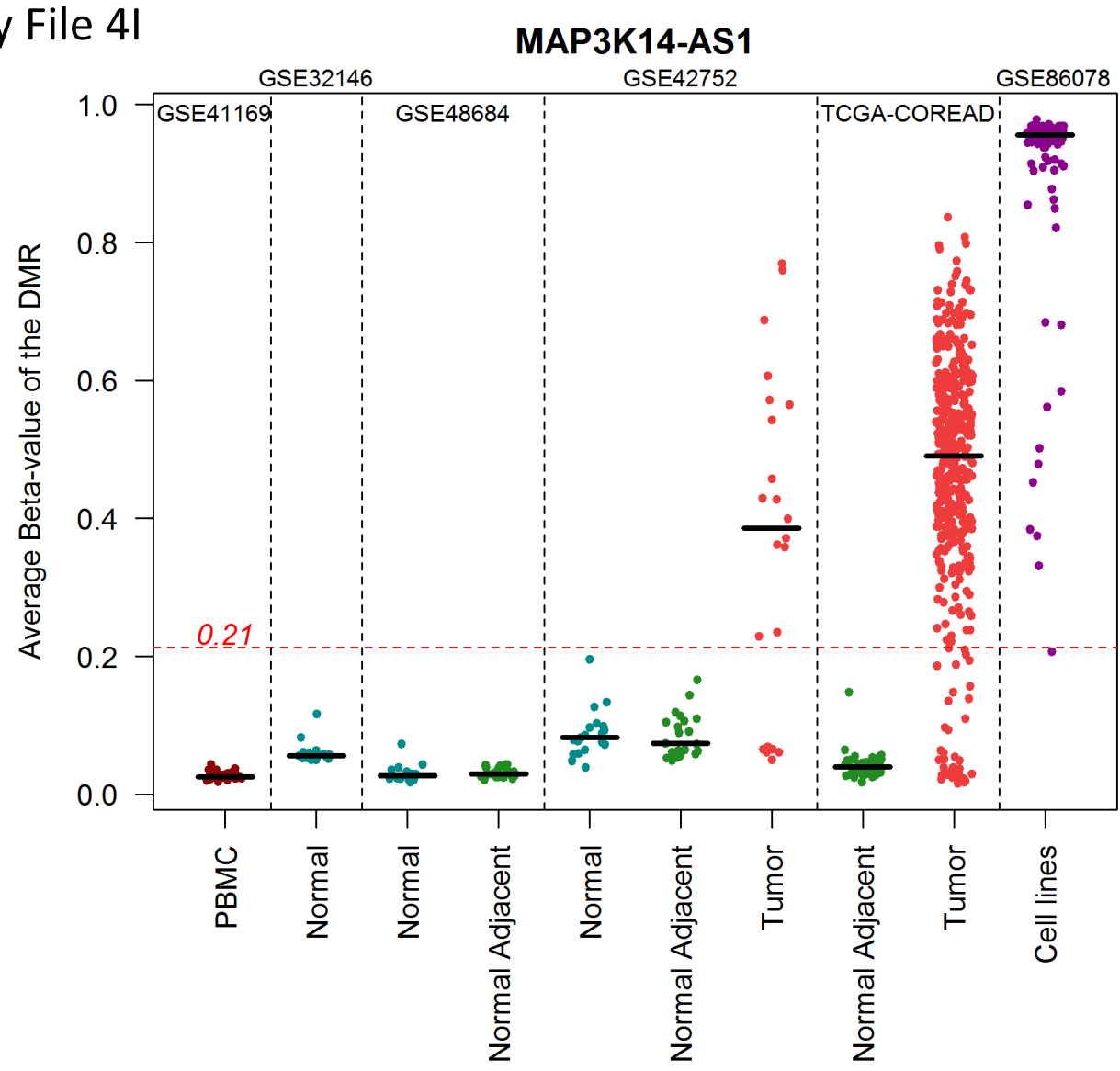
	<0.339	>0.339	NPV	PPV
Normal	45	0	0.7	1
Cancer	19	396		

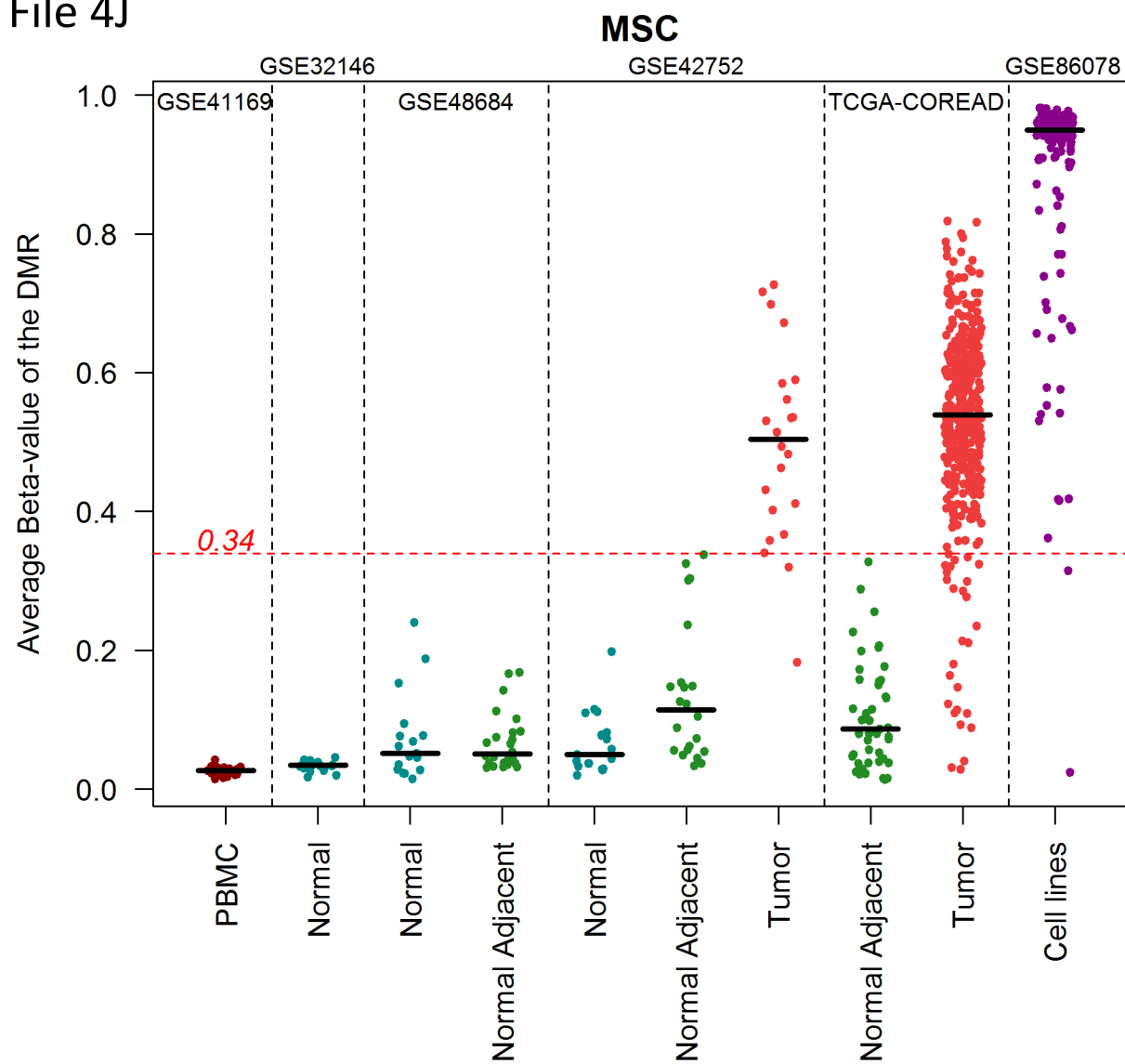




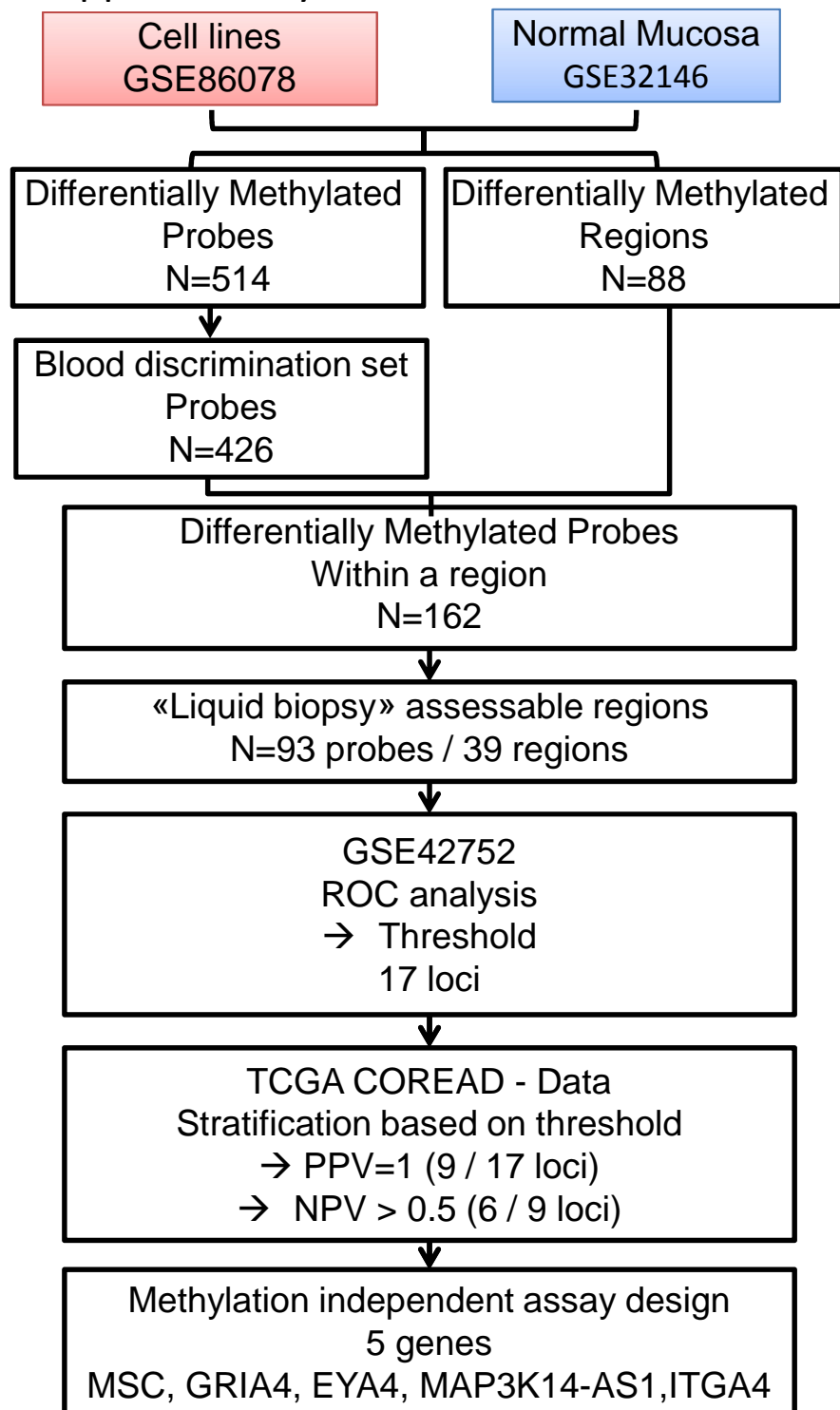
ITGA4







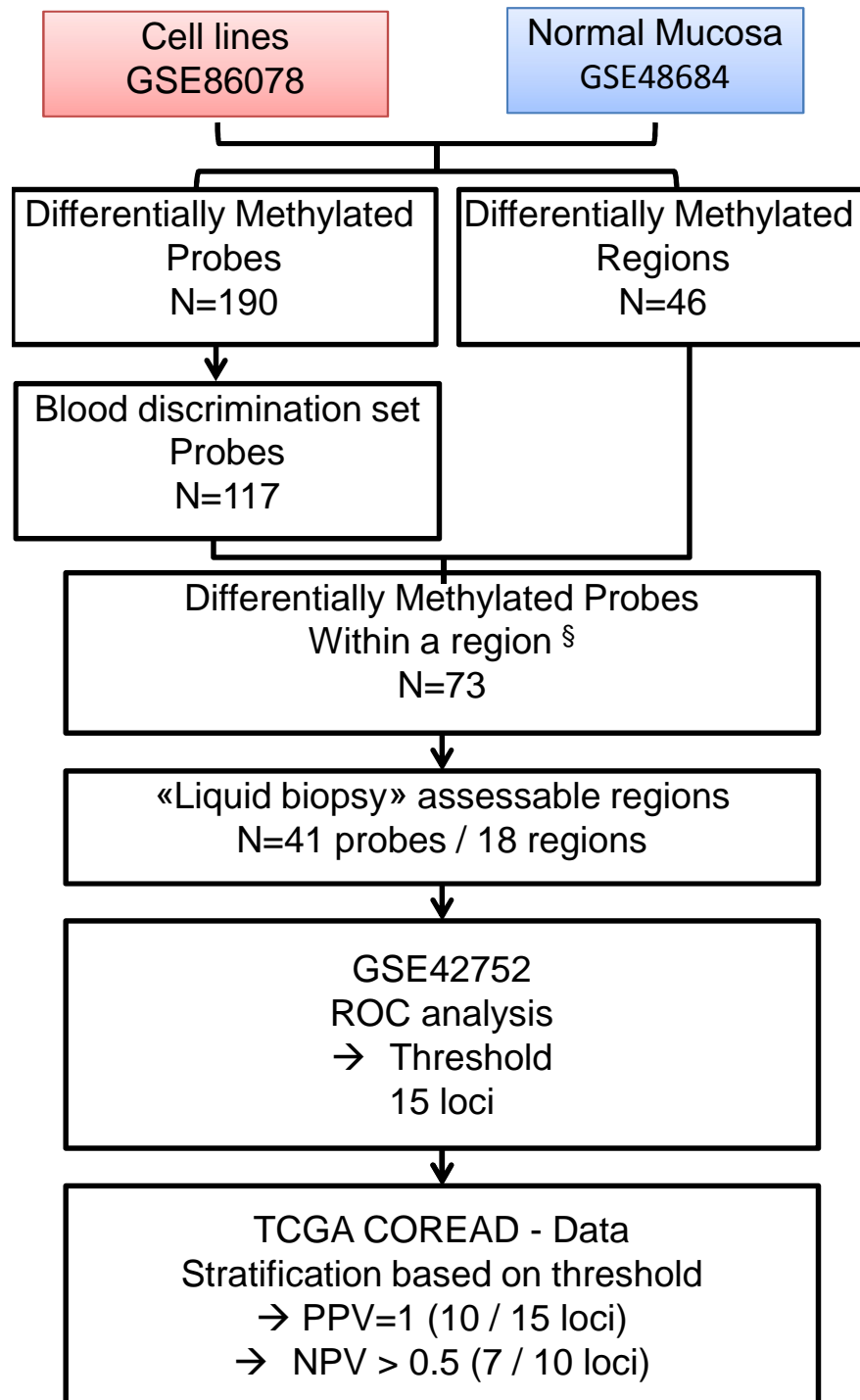
Supplementary File 5A



gene	seqnames	start	end	threshold	NPV	PPV
DAB1	chr1	58715539	58715553	0.42	0.500	0.997
SPAG6	chr10	22634432	22634439	0.31	0.495	1.000
KIAA1217	chr10	23983496	23983498	0.15	0.683	0.990
TLX1	chr10	102893980	102894120	0.38	0.600	1.000
INA	chr10	105036701	105036727	0.40	0.425	1.000
NELL1-A	chr11	20690720	20690807	0.27	0.829	0.974
NELL1-B	chr11	20691126	20691161	0.40	0.672	0.990
MIR129-2	chr11	43602845	43602879	0.36	0.592	1.000
GRIA4	chr11	105481317	105481322	0.26	0.714	1.000
DDX25	chr11	125774082	125774092	0.51	0.402	0.973
TM6SF1	chr15	83776269	83776420	0.27	0.543	0.997
BNC1	chr15	83952345	83952420	0.35	0.632	0.995
SALL1	chr16	51185001	51185082	0.38	0.542	1.000
C17orf46*	chr17	43339223	43339328	0.26	0.584	1.000
LOC100133991*	chr17	43339497	43339589	0.21	0.549	1.000
CLIP4-A	chr2	29338077	29338121	0.50	0.735	0.978
CLIP4-B	chr2	29338113	29338258	0.52	0.706	0.978
OTX1-A	chr2	63281069	63281139	0.20	0.409	1.000
OTX1-B	chr2	63283967	63284066	0.11	0.405	1.000
OTX1-C	chr2	63284066	63284132	0.13	0.415	0.997
CNRIP1	chr2	68546467	68546579	0.31	0.732	0.990
ITGA4	chr2	182322268	182322279	0.28	0.833	1.000
THBD	chr20	23029287	23029298	0.49	0.433	1.000
SLC32A1	chr20	37353096	37353126	0.45	0.423	0.997
TRH	chr3	129693489	129693586	0.42	0.657	0.997
PEX5L	chr3	179754603	179754613	0.41	0.412	0.992
TMEM155	chr4	122686453	122686493	0.40	0.579	0.997
MARCH11	chr5	16180033	16180076	0.47	0.254	1.000
SPOCK1	chr5	136834464	136834492	0.45	0.463	0.997
UBD	chr6	29521751	29521756	0.41	0.662	1.000
EYA4	chr6	133562461	133562492	0.23	0.714	1.000
SND1-A	chr7	127672473	127672564	0.30	0.977	0.995
SND1-B	chr7	127672564	127672658	0.34	0.977	0.995
FAM115A	chr7	143579665	143579698	0.53	0.652	1.000
NEFM	chr8	24772350	24772435	0.37	0.258	0.983
PLAG1	chr8	57069907	57070013	0.36	0.652	0.995
ADHFE1	chr8	67344665	67344720	0.46	0.714	1.000
TCF24	chr8	67874178	67874206	0.17	0.484	0.997
MSC	chr8	72756058	72756155	0.35	0.662	1.000

* same gene locus MAP3K14-AS1

Supplementary File 5B

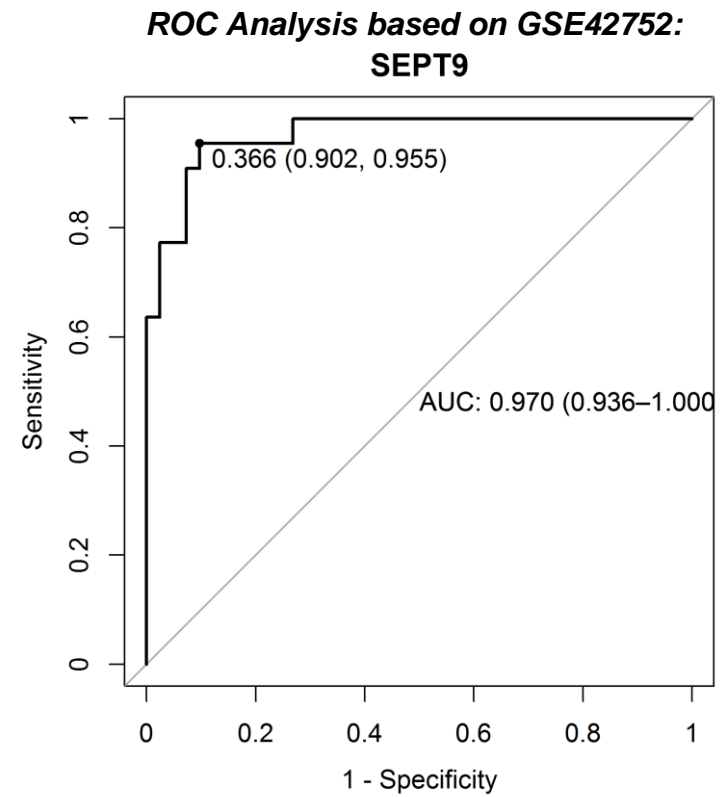
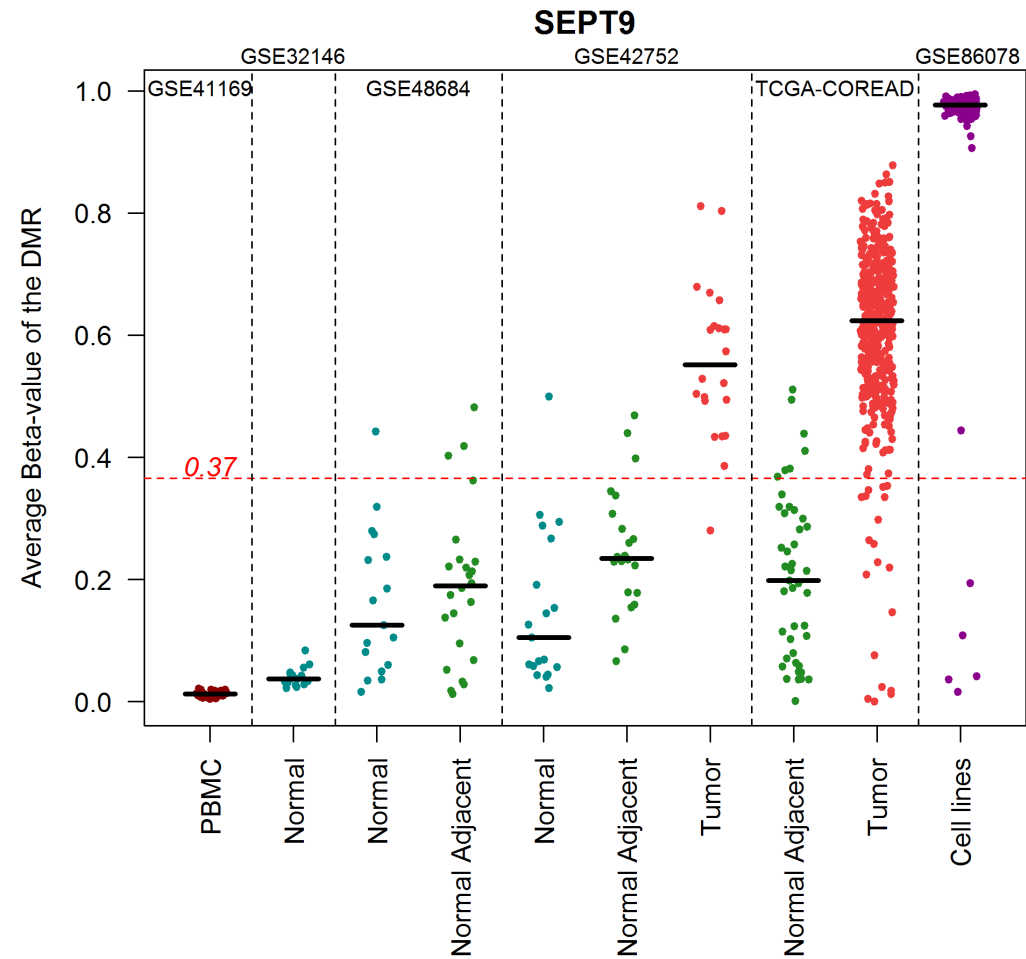


	seqnames	start	end	threshold	NPV	PPV
SPAG6	chr10	22634432	22634439	0.312	0.495	1.000
KIAA1217	chr10	23983496	23983498	0.148	0.683	0.990
GRIA4	chr11	105481317	105481322	0.258	0.714	1.000
C17orf46*	chr17	43339223	43339328	0.262	0.584	1.000
LOC100133991*	chr17	43339497	43339589	0.208	0.549	1.000
OTX1-A	chr2	63283967	63284066	0.114	0.405	1.000
OTX1-B	chr2	63284066	63284132	0.126	0.415	0.997
NXPH2	chr2	139537824	139537845	0.382	0.662	1.000
ITGA4	chr2	182322268	182322279	0.275	0.833	1.000
COL25A1	chr4	110223795	110223830	0.240	0.600	1.000
MARCH11	chr5	16180033	16180055	0.304	0.409	1.000
UBD-A	chr6	29521138	29521143	0.360	0.563	1.000
UBD-B	chr6	29521568	29521595	0.282	0.672	1.000
EYA4	chr6	133562479	133562492	0.199	0.738	1.000
SND1-A	chr7	127672169	127672235	0.095	0.657	0.997
SND1-B	chr7	127672473	127672564	0.304	0.977	0.995
ADHFE1	chr8	67344665	67344720	0.461	0.714	1.000
TCF24	chr8	67874178	67874206	0.169	0.484	0.997

* same gene locus MAP3K14-AS1

§ Loss of MSC region due to presence of only one significant DMP

Supplementary File 6A

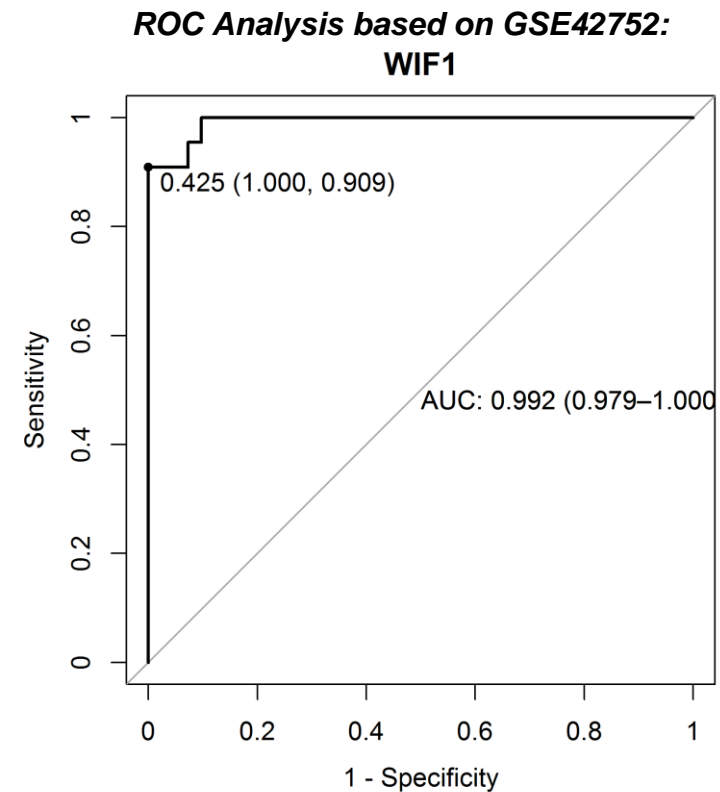
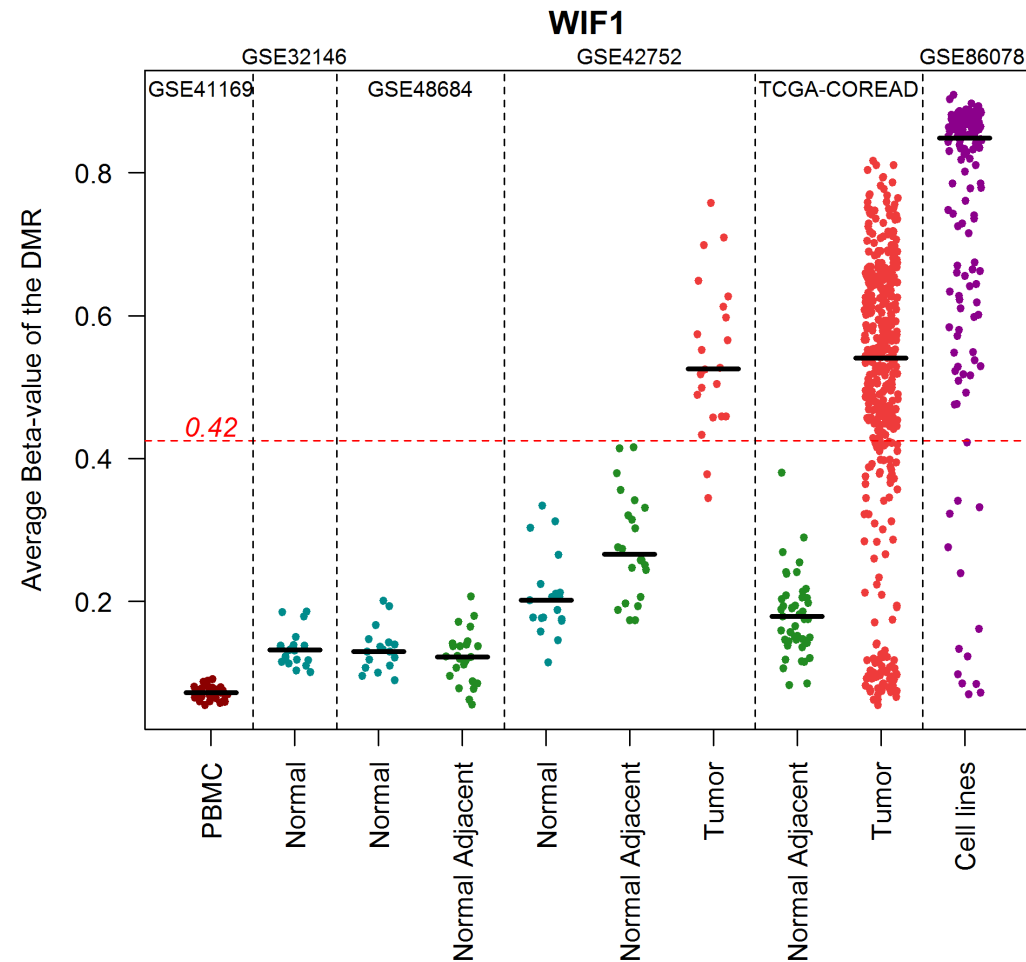


Region Assessed: Hg19: chr17: 75369484-75369657

Overlapping Probes: cg20275528; cg12783819

Exclusion criteria: DMP too far away >150bp.

Supplementary File 6B

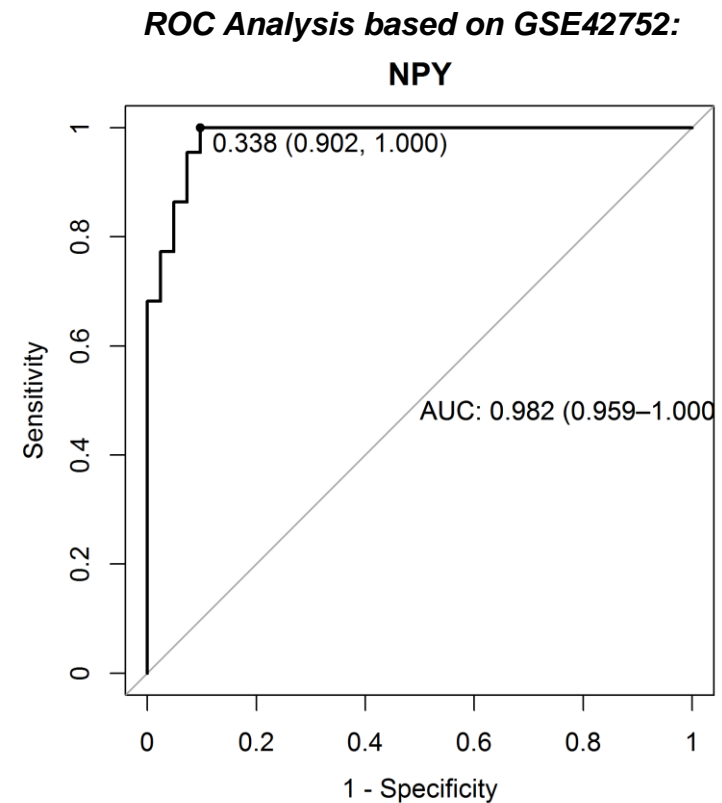
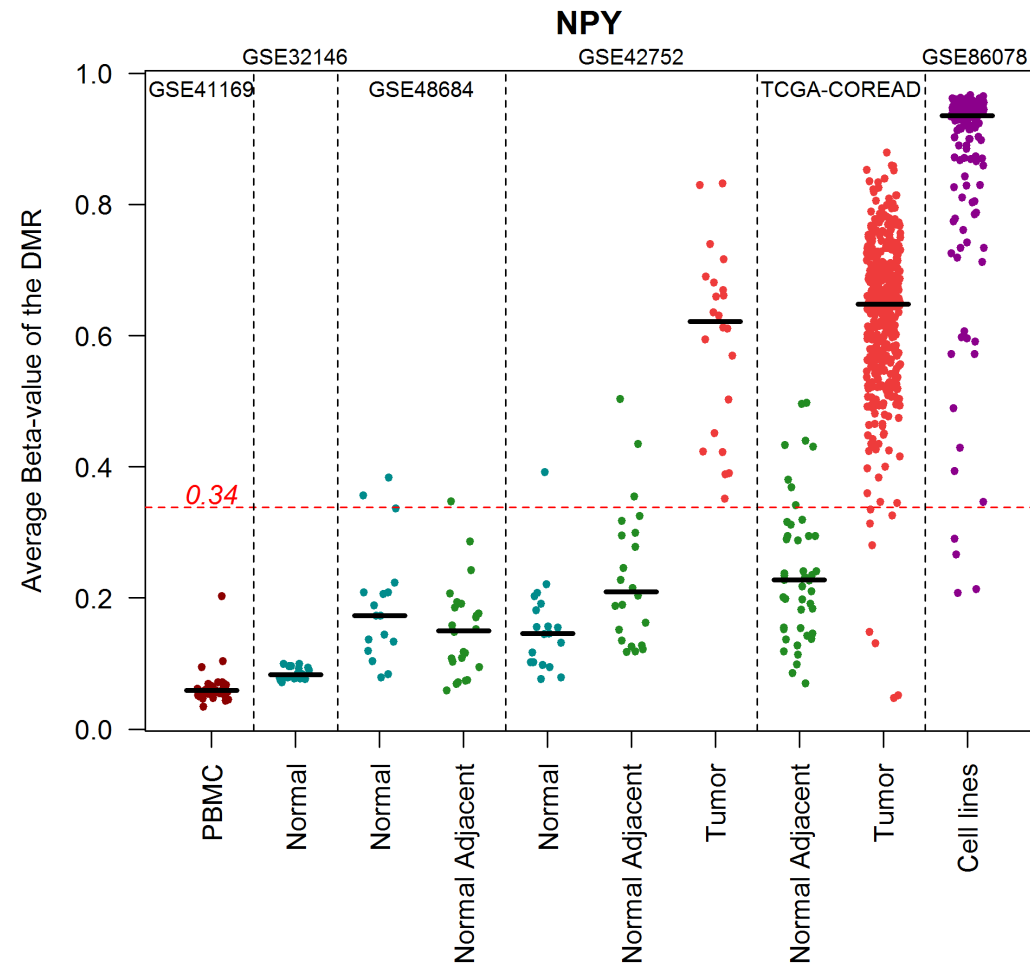


Region Assessed: Hg19:chr12:65514979-65515037 (assessed by Roperch *et al. BMC Cancer* 2013)

Overlapping Probes: cg03509412; cg19427610; cg26733786.

Exclusion criteria: differential methylation value below the 0.8 beta-value threshold; threshold established in GSE42752 above the 0.35 cut-off.

Supplementary File 6C

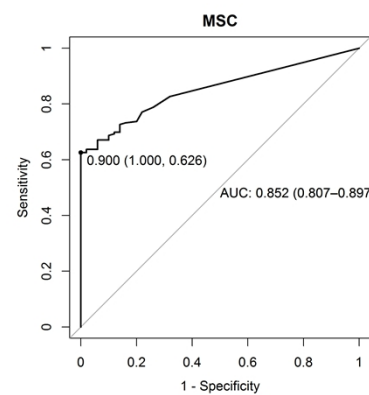
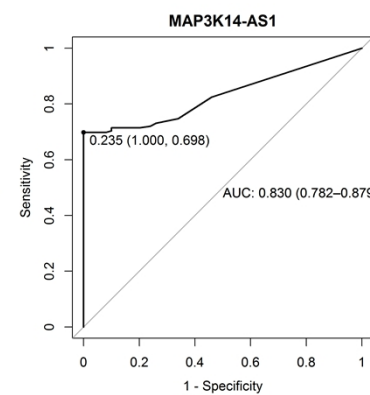
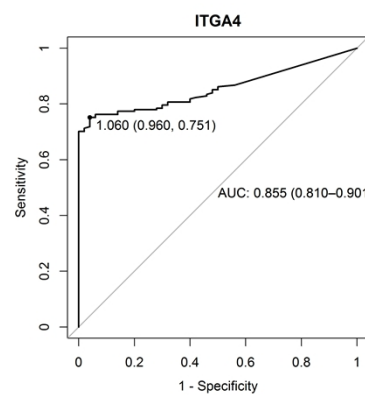
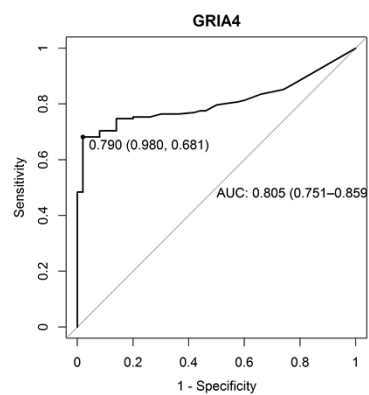
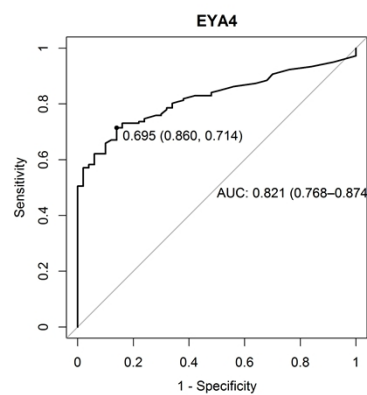
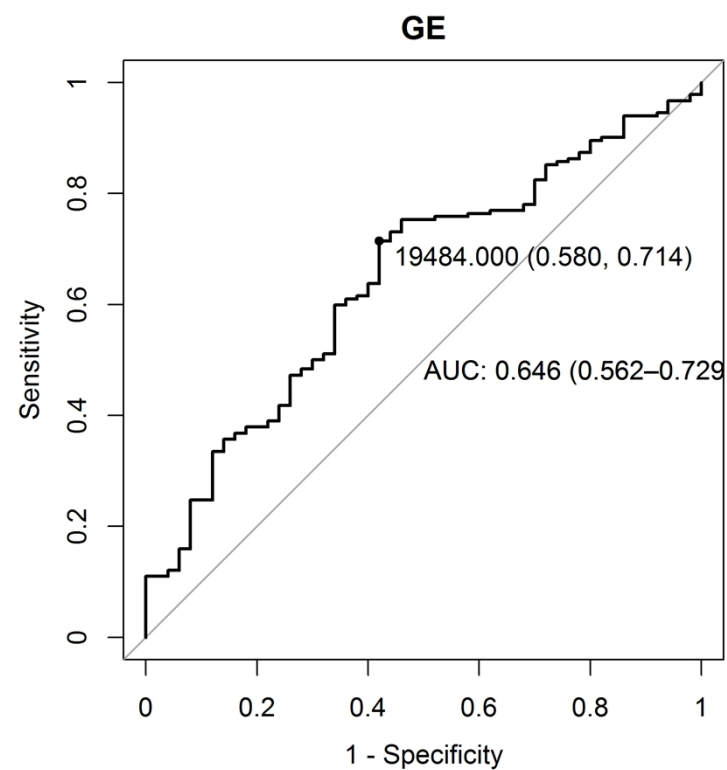
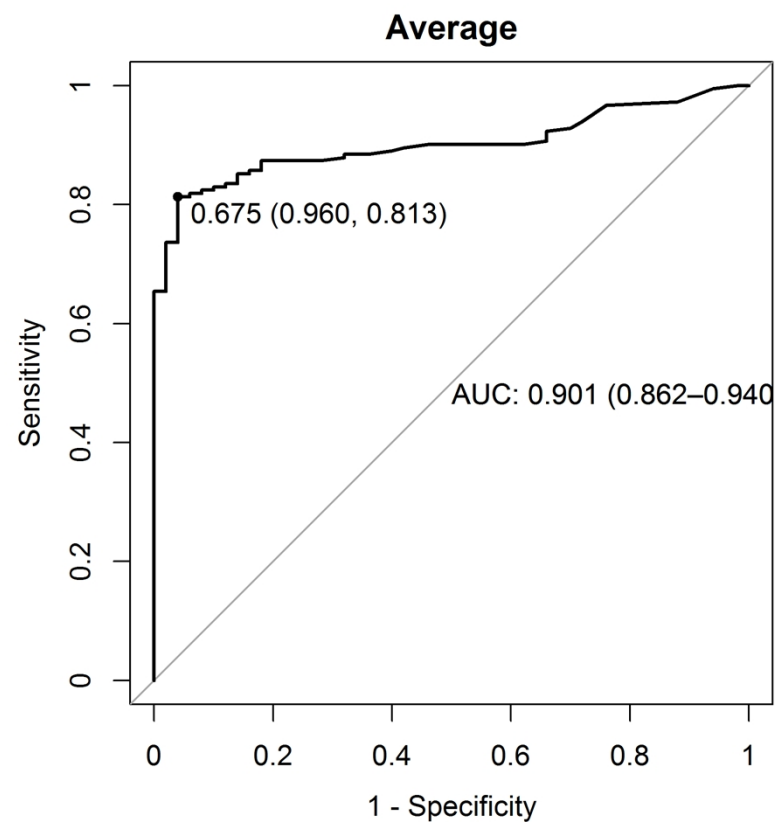


Region Assessed: Hg19:chr7:24323761-24323856 (assessed by Roperch *et al.* *BMC Cancer* 2013)

Overlapping Probes: cg24885417; cg00355281; cg15929698; cg16964348; cg25884711.

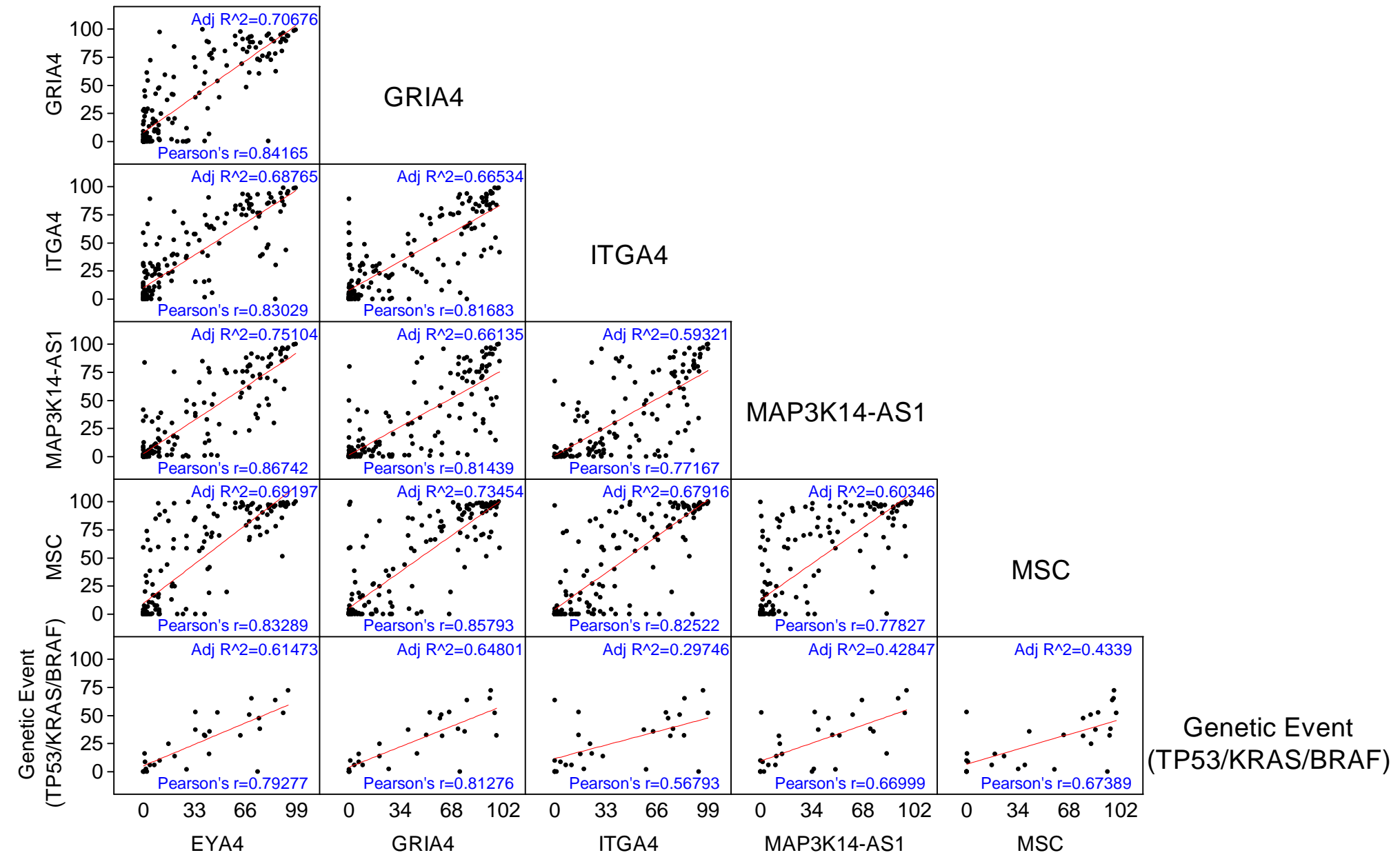
Exclusion criteria: positivity in blood (GSE41169) and multiple positive normal healthy and normal peritumoural tissue samples (yielding a specificity <1 in the validation cohort).

Supplementary File 7

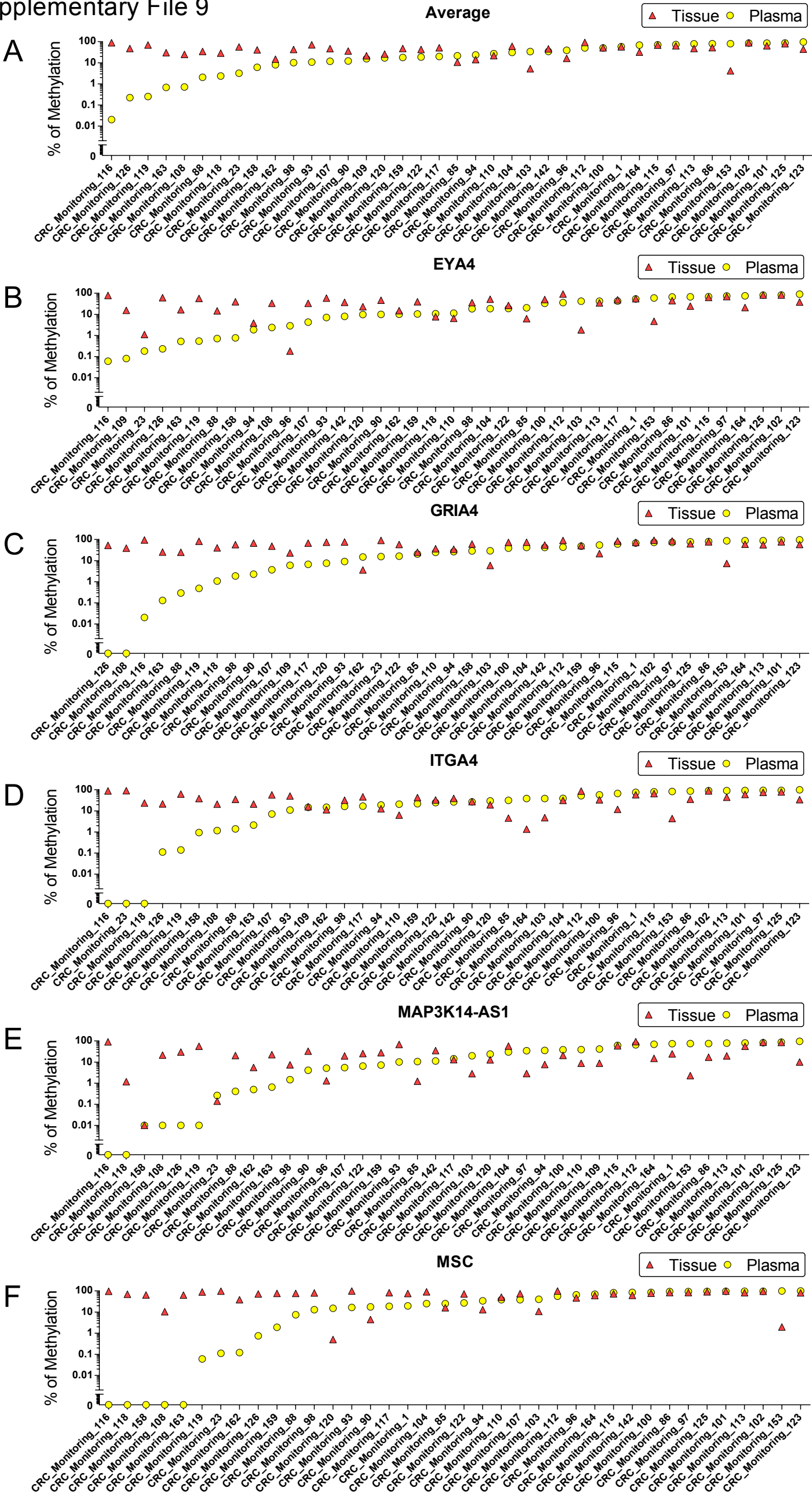


Supplementary File 8

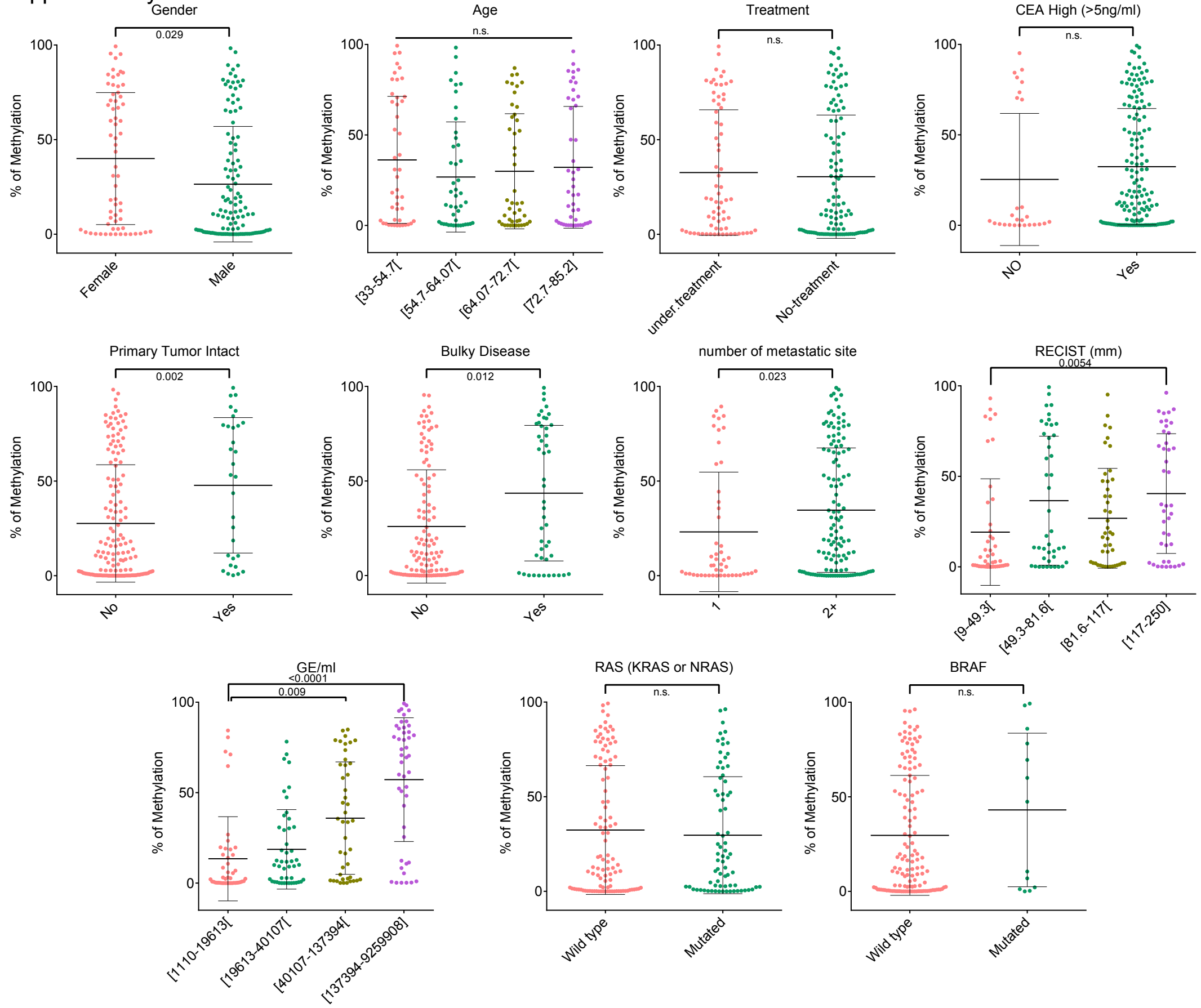
EYA4



Supplementary File 9



Supplementary File 10



Supplementary File 11

